

# Establishment, immunological analysis and drug prediction of a prognostic signature of ovarian cancer related to histone acetylation

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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

### *Author contribution statement*

Yujie Fang, Hecheng Wang, and Yanshuo Han conceived, designed, performed the study, and drafted the manuscript; Yujie Fang, Hao Zhang, Xu Guo, Jing Zhao, and Yanshuo Han analyzed the data; Yujie Fang, Hecheng Wang, Xiaoxu Zhang, and Yanshuo Han revised the manuscript. All authors read and approved the final manuscript.

### *Keywords*

histone acetylation, ovarian cancer, Prognostic Markers, targeted therapy, immune therapy

### *Abstract*

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In recent years, epigenetic modification has been increasingly regarded as an important hallmark of cancer. Histone acetylation, as an important part of epigenetics, plays a key role in the progress, treatment and prognosis of many cancers.

In this study, based on TCGA database, we performed LASSO regression and Cox algorithm to establish a prognostic signature of ovarian cancer associated with histone acetylation modulator genes and verified it externally in GEO database. Subsequently, we performed immunological bioinformatics analysis of the model from multiple perspectives using CIBERSORT algorithm, ESTIMATE algorithm and TIDE algorithm to verify the accuracy of the model. Based on the prognostic model, we divided ovarian cancer patients into high-risk and low-risk groups and assessed survival and the efficacy of accepting immunosuppressive therapy. In addition, based on the analysis of characteristics of the model, we also screened targeted drugs for high-risk patients, and predicted potential drugs that inhibit platinum resistance through connectivity map method.

We ultimately constructed a HAMs-related signature containing 10 histone acetylation modulators, among which HDAC1, HDAC10 and KAT7 can act as independent prognostic factors for ovarian cancer and are related with poor prognosis. In the analysis of the tumor microenvironment, the proportion of the B infiltrating cell and the macrophages was significantly different between the high and low risk groups. And the samples with high risk score had higher tumor purity and lower immune score. In terms of treatment, patients in the high-risk group who received immunotherapy had a higher likelihood of immune escape or rejection and were less likely to respond to platinum/paclitaxel therapy. Finally, we screened 20 potential drugs that could target the model for reference.

### *Contribution to the field*

In this study, we investigated the relationship between histone acetylation regulation and prognosis and treatment of ovarian cancer from the perspectives of genomics, targeted therapy and immune therapy. Firstly, Our study constructed a prognostic signature to predict the risk score of ovarian cancer patients, which can provide valuable reference for identifying high-risk groups of ovarian cancer and guidance for prognostic analysis of ovarian cancer patients. Subsequently, based on multiple analyses, we found that the risk score of patients was significantly correlated with the level of immune infiltration in patients, and there were distinct differences in TIDE scores and expression of immune checkpoints between subgroups, indicating that the model could predict the level of immunotherapy in patients to a certain extent. In addition, Our analysis found that high HDAC family expression was associated with poor risk score, which may be related to platinum resistance. And we have searched a number of promising potential targeted drugs for ovarian cancer patients. In a word, this research provides new idea for the prognosis and treatment strategy of ovarian cancer from the perspective of epigenetic modification and data analysis.

*Ethics statements*

*Studies involving animal subjects*

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In review

1 **Title Page**2       **Establishment, immunological analysis and drug prediction of a prognostic  
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31 **Abstract**

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34 modification, plays a key role in the progress, treatment and prognosis of many  
35 cancers.

36 In this study, based on TCGA database, we performed LASSO regression and  
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38 histone acetylation modulator genes and verified it externally in GEO database.  
39 Subsequently, we performed immunological bioinformatics analysis of the model  
40 from multiple perspectives using CIBERSORT algorithm, ESTIMATE algorithm and  
41 TIDE algorithm to verify the accuracy of the model. Based on the prognostic model,  
42 we divided ovarian cancer patients into high-risk and low-risk groups and assessed  
43 survival and the efficacy of accepting immunosuppressive therapy. In addition, based  
44 on the analysis of characteristics of the model, we also screened targeted drugs for  
45 high-risk patients, and predicted potential drugs that inhibit platinum resistance  
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48 acetylation modulators, among which HDAC1, HDAC10 and KAT7 can act as  
49 independent prognostic factors for ovarian cancer and are related with poor prognosis.  
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51 and the macrophages was significantly different between the high and low risk groups.  
52 And the samples with high risk score had higher tumor purity and lower immune  
53 score. In terms of treatment, patients in the high-risk group who received  
54 immunotherapy had a higher likelihood of immune escape or rejection and were less  
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56 drugs that could target the model for reference.

57 **Key words:** Histone acetylation; Ovarian cancer; Prognostic markers; Targeted  
58 therapy; Immune therapy

## 59 1. Introduction

60 Ovarian cancer (OC) is one of the three major malignant tumors of female  
61 reproductive system with the highest mortality rate<sup>[1]</sup>. Currently, more than 239,000  
62 new cases of ovary cancer occur worldwide each year (3.6% of all cancer cases),  
63 cause about 152,000 deaths each year (4.3% of all cancer deaths)<sup>[2]</sup>. The convert  
64 location of ovarian cancer in the pelvic cavity account for the inconspicuous  
65 symptoms, and most patients are in terminal stage when diagnosed due to the lack of  
66 effective screening methods. And the 5-year survival rate of patients with advanced  
67 stage is only 29%<sup>[3]</sup>. Tumor cell reduction and platinum-based chemotherapy are  
68 usually the initial treatment for ovarian cancer, but 70% of patients with epithelial  
69 ovarian cancer will relapse within 3 years<sup>[3]</sup>. And multiple relapses lead to increased  
70 resistance to chemotherapy drugs through a bewildering array of mechanisms.

71 Studies have shown that the progression and treatment effect of OC are affected  
72 by many factors such as disease classification and staging, treatment strategy and  
73 tumor microenvironment. Many transcriptional and epigenetic studies have also  
74 demonstrated that the occurrence, progression and prognosis of OC are affected by  
75 the dynamic changes of multiple oncogenes and tumor suppressor genes<sup>[6]</sup>. A few  
76 genes that may predict the prognosis of OC have been found in previous studies, but  
77 their clinical application is relatively limited.

78 Histone acetylation is a dynamically reversible process that determines the loose  
79 state of chromatin, and the relaxed chromatin in the acetylation state facilitates gene  
80 transcription, normally<sup>[7]</sup>. The dynamic process of histone acetylation is controlled by  
81 a series of histone acetylation modulators (HAMs), which can be classified as Writers,  
82 Erasers, and Readers. Writers include histone acetyltransferases (HATs), which

regulate gene transcription by adding acetyl groups to lysine residues of H3 or H4. Acetylation of histones is also removed by histone deacetylases (HDAC), a class of enzymes known as Erasers. In addition, proteins called histone acetylation readers recognize acetylated histones and recruit transcriptional mechanisms<sup>[8]</sup>. These proteins generally contain bromodomain (BRD) or are themselves acetyllysine-binding proteins, such as the Bromodomain and extra-Terminal domain (BET) family. They are Readers that specifically bind acetylated histone H3/H4 and recruit downstream effectors to activate transcription<sup>[9]</sup>. Readers identify lysine residues at the tail of acetylated histones by BRD domain. This recognition is a prerequisite for protein-histone association and chromatin remodeling and is closely related to transcriptional activation<sup>[10]</sup>.

As an important part of epigenetic modification, histone acetylation plays an iconic role in the occurrence, development and prognosis of many cancers. Unexpected high frequency mutations in genes involved in the regulation of histone acetylation have been found in many cancers in recent genomic studies, suggesting that some HAMs may act as drive genes in cancer development<sup>[10]</sup>. In epigenetic studies of breast cancer, samples with higher levels of acetylation of H4 showed a better prognosis, and showed an overall decrease in normal breast epithelium compared with breast cancer tissue, suggesting that acetylation regulation has an impact on the prognosis of cancer<sup>[12]</sup>.

In recent years, targeted therapy and immunotherapy have become the key methods in the treatment of many advanced cancers due to their advantages of small toxicity and strong targeting<sup>[13]</sup>. In terms of gene-targeted therapy, dysregulation of transcription due to altered protein acetylation patterns is a hallmark of cancer, which is currently a mechanism by which HDAC inhibitors are targeted<sup>[14]</sup>. Presently, there are three HDAC inhibitors available for clinical treatment of ovarian cancer, and there are many targeted drugs for acetylation in preclinical trials, and more targeted drugs

110 for histone acetylation are waiting to be discovered<sup>[15]</sup>. In terms of immunotherapy,  
111 many immune checkpoint inhibitor drugs (ICI) has been in the treatment of  
112 cancer. But only a small number of patients can benefit from it due to the specificity of  
113 immunotherapy drugs. Besides, some cancers, such as pancreatic cancer, breast cancer  
114 or ovarian cancer, seems to has intrinsic resistance to ICI drugs<sup>[16]</sup>. How to identify the  
115 population that responds to immunotherapy drugs is a current problem.

116 In this study, we collated genes involved in acetylation regulation, established a  
117 novel prognostic signature associated with acetylation regulation using samples from  
118 TCGA and GTEx databases, and classified cancer patient populations into high-risk  
119 and low-risk groups. Based on CIBERSORT, ESTIMATE, and ssGSEA algorithms,  
120 we discuss differences in immune and survival characteristics among different risk  
121 subgroups in tumor immune infiltration. We focused on the expression of symbolic  
122 genes CA125 and HE4 and immune checkpoint genes PD-1, PD-L1, PD-L2 and  
123 CTLA4 in different groups of ovarian cancer, so as to further explore the correlation  
124 between histone acetylation and the progress, treatment and prognosis of ovarian  
125 cancer. To evaluate the role of risk subgroups in the treatment of ovarian cancer,  
126 IMVIgor210 and GSE30161 were introduced for validation. Further, we used the  
127 MOA(mode of action) method in cMAP database to screen out potential drugs  
128 targeting the model. In addition, gene functional enrichment analysis and protein  
129 interaction network were used to improve the interaction mechanism of model genes.  
130 In conclusion, our study established a link between the expression of histone  
131 acetylation modulators and the progress, therapy and prognosis of ovarian cancer,  
132 providing new ideas for the prognosis and treatment of ovarian cancer, and providing  
133 help for ovarian cancer patients to find more effective targeted drugs.

134 **2. MATERIALS AND METHODS**

135 **2.1Data source**

136 RNA-seq data of 375 serous ovary cancer patients (the recurrence samples have

been removed) and corresponding clinical information were from the TCGA-OV data set (data version:07-20-2019).RNA-seq data of 88 normal ovarian tissue samples was from the GTEx data set (data version:04-19-2016)(<https://xenabrowser.net/datapages/>). The expression microarray data and clinical information of 260 serous ovarian cancer patients from GSE32062 were downloaded at GEO database<sup>[17]</sup>. The data format of the expression matrix adopt TPM (transcripts per kilobase million), and the standardized method is log2(TPM+1).R package limma and sleuth were used for further quality control and data collation, and the average value of gene expression level was adopted for Multi-probe genes.

A total of 77 acetylation regulatory genes were straightened out refer to the literature<sup>[11]</sup>, including 22 histone acetylation genes(Writers), 18 histone deacetylation genes (Erasers), and 43 histone acetylation recognition genes (Readers), among which 6 genes serve as both Writer and Reader.(Supplementary Table 1)

## 2.2Identification and enrichment analysis of differentially expressed HAMs

Differentially expressed HAMs were analyzed by limma package in R software<sup>[18]</sup>. To further investigate the biological functions of differential genes, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted utilizing GOpot package in R software.

## 2.3Construction and validation of the HAMs-related signature for patients with ovary cancer

R package glmnet was applied to perform the LASSO regression algorithm to train the Regression coefficient<sup>[19]</sup>.Optimal  $\lambda$  value was determined by 10-fold cross-validation and 1se analysis. Then the model genes were further screened by Akaike information criterion(AIC), and a gene signature containing 10 HAMs was established by multivariate Cox regression. The risk score was obtained by the following formula:

163 Riskscore=Σ (Regression coefficients × Level of gene expression)  
164 Therefore, TCGA ovarian cancer patients can be divided into high-risk low-risk  
165 groups according to the median risk score.

166 To further verify the accuracy of the signature,Kaplan-meier curve was drawn by  
167 survival package of R, and Area Under Curve(AUC) of time-dependent ROC curve  
168 was analyzed by timeROC package<sup>[20]</sup>, and a nomogram of OS prediction probability  
169 was established by rms package as internal verification. And the GSE32062 dataset is  
170 acted as an external validation cohort. Risk scores of 260 OC samples were calculated  
171 using the above formula, and the KM curve and ROC curve were also used to verify  
172 the performance of the gene signature. Additionally, we reviewed previous literature  
173 on marker construction for ovarian cancer to ensure that AUC values had prognostic  
174 credibility<sup>[6, 21-24]</sup>.

175

#### 176 **2.4The correlation between gene expression and immune infiltration**

177 Cibersort is a convolution tool for expression matrix of immune cell subtypes  
178 based on linear support vector regression. CIBERSORT source was downloaded(<https://cibersort.stanford.edu/>) and was then performed in the R platform. The  
179 whole-gene expression matrix of GTEx and TCGA was input to predict the  
180 relative proportion of 22 kinds of immune cells in the sample and the expression  
181 of immune cells between the high-risk group and the low-risk group was  
182 compared. R package estimate was applied to predict the immune score and tumor  
183 purity of samples and to compare them in high and low risk groups.

185 In addition, we downloaded immune estimation data of TEGA samples from the  
186 TIMER database<sup>[25]</sup>. (<https://cistrome.shinyapps.io/timer/>). The relationship between the relative weight of immune cells(CD4+ T cells, CD8+ T cells, B cells, macrophages) and risk score was explored and the correlation chart was drawn.

190

191       **2.5Therapeutic effect evaluation**

192       In order to evaluate the response of different subgroups of patients to Im  
193       mune Checkpoint Inhibitors(ICIs), we first compared the expression levels of fo  
194       ur important immune checkpoint genes. Further, TIDE algorithm was used to e  
195       valuate the possibility of each patient's tumor immune escape<sup>[26]</sup>.(<http://tide.dfci.harvard.edu/>).  
196

197       IMVIgor210 were the immunotherapy cohorts introduced as external validat  
198       ion cohort to verify the consistency of immunotherapy effect and prediction<sup>[27, 2</sup>  
199       <sup>8]</sup>.GSE30161 were used to study the relationship between the model and platin  
200       um resistance, which contains 58 patients with ovarian cancer receiving platinu  
201       m chemotherapy<sup>[29]</sup>.

202

203       **2.6Mutation analysis**

204       Mutation profile of OC samples were derived from the TCGA database. T  
205       he R package maf-tools is used to process and analyze data in MAF format. W  
206       e visualized the frequency of mutations in the high/low risk groups and calcula  
207       ted theTumor Mutation Burden(TMB) score for each sample: TMB = (total mu  
208       tations/total covered bases) × 10<sup>6</sup>.

209

210       **2.7Prediction of potential target compounds for OC patients**

211       We utilized Broad's CMap database to predict potential drugs that target the  
212       HAMS-related signature<sup>[30]</sup>.The mode of action(MoA) analysis was used to sort out  
213       the class and mechanism of drugs.

214

215 **2.8Identification of crucial prognostic HAMs**

216 Survival analysis was used to explore the prognostic value of signature genes and  
217 to identify genes with independent prognostic ability. Subsequently, protein  
218 expression of these independent prognostic factors was confirmed in the Human  
219 Protein Atlas(HPA)database(<http://www.proteinatlas.org/>)<sup>[31]</sup>.

220

221 **2.9Protein expression analysis**

222 The STRING database and the geneMania database were used to build the  
223 protein-protein interaction(PPI) network. The STRING database (<https://string-db.org>)  
224 depicts a network of physical and functional interactions of proteins based on  
225 systematic co-expression analysis and literature text mining<sup>[32]</sup>. PPI network analysis  
226 was then constructed to predict physical and functional interactions of prognostic  
227 HAMs to explore core genes of the network. GeneMANIA  
228 database(<http://www.genemania.org>) can be used to identify genes associated with  
229 signatures for further analysis<sup>[32]</sup>.Interaction networks associated with signature genes  
230 were constructed by identifying gene co-annotation patterns in gene ontology or using  
231 enrichment analysis.

232

233 **2.10Statistical analysis**

234 In this study, all statistical analyses were conducted using Perl software (version  
235 5.32.1.1) and R software (version 4.1.1).Wilcoxon test and Kolmogorov-Smirnov test  
236 were used to compare paired groups.Besides, p value <0.05 was considered as  
237 statistically significant.

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240     3. RESULT

241       **3.1Differential Expression Analysis of HAMs in OV**

242       The workflow of our study is illustrated in Figure 1. Firstly, we downloaded  
243 mRNA data of 375 OV samples and 88 corresponding normal ovary samples with  
244 clinical information from the TCGA and GTEx database. By comparing the gene  
245 expression profiles in the TCGA cancer group and the GTEx normal group, a total of  
246 21 differentially expressed genes(DEGs) were identified among 77 HAMs  
247 genes( $|logFC|>1, p<0.001$ ), with 8 downregulated genes and 13 upregulated  
248 genes(**Figure 2A,2B**). Compared with normal group, the expression of SP110 and  
249 HDAC10 reached -2.70 and -2.30 logFC respectively, which were significantly  
250 down-regulated. In contrast, the expression of ATAD2 and ZMYND8 were  
251 up-regulated with logFC of 1.96 and 1.98, respectively. All differential expression  
252 results are presented in supplementary materials. (**Supplementary Table 2**)

253

254       **3.2GO and KEGG functional analysis**

255       Through GO enrichment analysis, the differentially expressed genes were mainly  
256 enriched to 77 items( $q\text{-value}<0.05$ ), including Histone modification, Peptidyl-Lysine  
257 modification, Histone binding, Histone deacetylase activity, etc(**Supplementary**  
258 **Table 3**). The results of KEGG analysis showed that HAM differential genes were  
259 mainly enriched in three pathways( $q\text{-value}<0.05$ ): Viral carcinogenesis, Alcoholism,  
260 Neutrophil extracellular trap formation(**Supplementary Table 4**) (**Figure 2C-2F**).

261

262       **3.3Development of the HAMs-related signature**

263       TCGA cohort(TCGA-OV, n=375) is used as a training set to construct  
264 HAMs-related signature. To eliminate overfitting of gene signature, LASSO

265 regression was performed to screen genes and train regression coefficients. Then,  
266 genes that contributed less to the model were filtered out by AIC criterion. Finally, a  
267 10-gene prognostic marker was obtained, and the formula was as follows:

268  $\text{Riskscore} = (-0.3818) * \text{ELP3} + (0.2097) * \text{HDAC1} + (0.2688) * \text{HDAC10} + (0.2029) * \text{H}$   
269  $\text{DAC11} + (0.1501) * \text{HDAC2} + (0.3085) * \text{HDAC4} + (0.4347) * \text{KAT7} + (-0.3345) * \text{KIAA202}$   
270  $6 + (-0.4711) * \text{SIRT5} + (-0.1796) * \text{SP140}$

271 According to the calculated median Risk score, TCGA patients were then divided  
272 into high-risk and low-risk groups(**Supplementary Tables 5**).The hazard ratio of the  
273 signature were presented (**Figure 3A**).As shown in Figure 3A,HDAC1, HDAC2,  
274 HDAC4, HDAC10, HDAC11, and KAT7 genes in the HDAC family were bad  
275 prognostic factors (Hazard ratio>1), while ELP3, KIAA2026, SP140, and SIRT5 were  
276 good prognostic factors (Hazard ratio< 1). Further more, the line diagram and  
277 calibration diagram of the model were also performed (**Figure 3B,3C**).

278 The time-dependent ROC curve analysis and Kaplan-Meier curves were  
279 performed (Figure 3D, 3E) for internal validation of the model. The area under the  
280 curve(AUC) values of HAMs-related signature at 1,3,and 5 years were 0.737,0.704  
281 and 0.688,respectively. In order to achieve a horizontal comparison, a table includes  
282 several previous modeling of prognostic signature for ovarian cancer were  
283 compiled(**Supplementary Table 6**).

284

### 285 **3.4External validation of the HAMs-related signature**

286 GSE32062 (n=260) is a large ovarian cancer dataset from the GEO database,  
287 which is used for external validation of the gene signature. Consistent with internal  
288 validation,the Kaplan-Meier curves (**Figure 4A**) showed that the high-risk group had  
289 poorer prognosis.The time-dependent ROC curve analysis was conducted and the  
290 AUC values 0.66, 0.584, and 0.638 of 1,3,5-years of prognostication, respectively  
291 (**Figure 4B**).

292

293       **3.5Comparing the immune infiltration between the subgroups**

294       Tumor immune infiltration is one of the main biological characteristics of  
295       various cancers and is significantly related to the prognosis.To study the inner  
296       relationship between immune infiltration and the gene signature, we predict the  
297       distribution of 22 immune cells in TCGA and GTEx cohort by CIBERSORT  
298       algorithm at first. The heat map and violin diagram (**Figure 5A,5B**) indicates that the  
299       distribution of macrophages, B cells and CD4 cells have significant differences  
300       between tumor tissues and normal tissues.

301       Next, we compared the immune infiltration of TCGA samples in the high- and  
302       low-risk group (**Figure 5C**).It shows that the B memory cells and macrophage M1  
303       were significantly different between high- and low-risk group, indicating a correlation  
304       with prognosis.Further, the relationship between six immune cells and the Risk score  
305       was comprehensively compared (**Figure 5D**).Thereinto,the infiltration level of CD8  
306       cells, dendritic cells, and neutrophils were negatively correlated with the Risk score,  
307       while the macrophages were significantly correlated with the higher risk.

308       Subsequently, we applied ESTIMATE algorithm to calculate the immune score,  
309       estimate score and tumor purity between low-risk and high-risk groups (**Figure**  
310       **5E**).As shown in Figure 5E,patients with high risk score had significantly lower  
311       immune scores than those in the low-risk group, while tumor purity was significantly  
312       higher compared to the low-risk group.

313

314       **3.6Evaluation of immune status between the subgroups**

315       By comparing the gene expression profiles of TCGA patients, we found that the  
316       expressions of CTLA4, PD-L1 and PD-L2 were significantly different among  
317       different risk groups, and the expression levels were higher in the low-risk group

318 (**Figure 6A**). Applying TIDE algorithm, we calculated Tidesore, Exclusion score and  
319 Dysfunction score of each sample, and the scores of all three were high in the  
320 high-risk group. In addition, the infiltrating results of Myeloid-derived suppressor  
321 cells (MDSCs) and the M2 subtype of tumor-associated macrophages (TAM.M2) and  
322 the expression of interferon -  $\gamma$  (IFNG) were significantly different between the  
323 different risk subgroups (**Supplementary Figure 3**) and correlated with the risk  
324 score(**Figure 6B**).

325 To further explore the role of the risk score model in predicting the immune  
326 response of patients, we introduced the IMVIgor210 cohort for  
327 analysis(**Supplementary Table 7**) . With the increase of risk score, the immune state  
328 of patients changed from inflamed to excluded and desert (**Figure 6C**), and the degree  
329 of immune infiltration decreased periodically. In addition, patients with high risk  
330 score had a lower objective response rate (ORR) to ICI than those in the low-risk  
331 group. (**Figure 6D**)  
332

### 333 **3.7Mutation profile and HAMs risk groups**

334 Gene mutation is one of the main reasons of tumor occurrence and progre  
335 ss. By evaluating the frequency of tumor mutation, the Tumor Mutation Burden  
336 (TMB) of patients can be calculated. According to the model established, prop  
337 ortion order of somatic mutations in high-risk group was as follows: TP53>TT  
338 N>CSMD3>NF1>USH2A >MUC16(CA125) >TOP2A > MACF1>FLG>LAM (**Fi**  
339 **gure 7A**), and in the low-risk group: TP53>TTN>CSMD3>MUC16(CA125) >  
340 RYR2 > FAT3 > DST >MYH4 > BRCA1 > MUC17 (**Figure 7B**).TMB is an  
341 important indicator currently used to evaluate immunotherapy, clinically. Compa  
342 red with patients with high risk score, TMB was significantly increased in the  
343 low-risk group, and TMB was correlated with patient survival (**Figure 7C,Figu**  
344 **re 7D**).

---

345346       **3.8Targeted therapy analysis and drug prediction**

347       Subsequently, based on the GSE30161 dataset, we compared the prognosis  
348       of patients receiving platinum/paclitaxel with different level of risk scores(**Sup**  
349       **plementary Table 8**). The result showed that the high-risk group had a lower  
350       percentage of complete response (CR) after treatment. (**Figure 8A,8B**)

351       By comparing gene expression characteristics between patients in the high  
352       and low risk groups(**Supplementary Table 9**), we used MoA analysis of CMap  
353       to identify 20 ideal compounds for targeting genetic signature (**Figure 8C**). T  
354       he mechanisms of these drugs include HDAC inhibitor, CDK inhibitor, PLK in  
355       hibitor, mTOR inhibitor, MEK inhibitor, etc. Among them, HDAC inhibitors se  
356       rves as the main pathway towards the signature consist of 6 drugs:ISOX (Scor  
357       e=96.26), APicidin (Score=96.09), WT-171 (Score=94.31), Vorinostat (Score=92.  
358       59), THM-I-94(Score=88.99), Trichostatin-a (Score=87.17).(**Table 1**)

359

360

361       **3.9Exploring crucial independent prognostic HAMs**

362       Survival analysis was applied to identify the prognostic value of HAMs signature  
363       genes in TCGA cohort. Only HDAC1( $p=0.028$ ),HDAC10( $p=0.035$ ) and  
364       KAT7( $p=0.002$ ) were tested for significant survival correlations(**Figure 9A**).In  
365       addition,lower expression of HDAC1, HDAC10, and KAT7 resulted in a relatively  
366       longer survival, while SP140 ( $P =0.064$ ) was detrimental to survival.(**Supplementary**

367       **Figure 4**)

368

369

370      **3.10 Protein expression analysis of crucial HAMs**

371      The immunohistochemical diagram of the HPA database was presented(**Figure**  
372      **9B**).The results showed that the protein expression level of HDAC10 was  
373      significantly down-regulated in tumor samples.Moreover,the protein expression levels  
374      of HDAC1 and KAT7 were also down-regulated to a certain extent, which was  
375      consistent with the results of survival analysis.

376      Based on STRING and geneMania database,we have explored the protein  
377      interaction network of the signature genes(**Figure 9C,9D**).The STRING database  
378      shows the inner interaction network.The result indicates that CREBBP, HDAC1 and  
379      HDAC2 possess the most internal interactions.Further,through protein interaction  
380      analysis conducted by geneMania database, we found some genes associated with  
381      HAMs signature, such as HDAC9, HDAC8, ALL133500.1, HDAC6, AGMAT, etc.  
382

383      **4. DISCUSSION**

384      Ovarian cancer is one of the diseases with the highest mortality rate of  
385      gynecological malignant tumor<sup>[34]</sup>. The lack of effective and sensitive diagnostic  
386      means in early stage of ovarian cancer, and the cancer has chemotherapy resistance  
387      and metastasis in advanced stage, resulting in poor treatment effect and prognosis of  
388      patients<sup>[35]</sup>. Therefore, rapid and accurate early diagnosis and rational medication and  
389      treatment strategies are the key to the treatment of ovarian cancer. Genetics and  
390      epigenetics are two key factors that determine the occurrence and development of  
391      tumors. A large number of epigenetic modification related genes are changed at a high  
392      frequency in cancer and may become driving genes in the process of cancer  
393      development<sup>[11]</sup>. Histone acetylation, involved in the regulation of cell cycle, cell  
394      differentiation and apoptosis, greatly affects the occurrence, development and  
395      treatment of cancer<sup>[36]</sup>.

396      In this study, we studied 77 important HAMs, including Wirter, Eraser and

Reader. Through GO and KEGG enrichment analysis, these HAMs are mainly involved in the modification of histones and the regulation of a variety of transcriptional activities, thus playing an important role in the progress, development and prognosis of cancer. By analyzing the transcriptional expression profile of HAMs of ovarian cancer patients in TCGA and normal samples in GTEx, we established a prognostic signature of ovarian cancer associated with HAM genes and verified it in the GEO database. According to the risk score of our model, patients can be divided into high-risk and low-risk subgroups, and the OS of patients in the two subgroups is significantly different in both the training cohort and the validation cohort. The results of ROC curve and nomogram indicate that the risk model established by us is effective in prognosis.

Among the multi-gene signature established by us, there are 10 HAMs, among which 2 belong to histone acetylation enzyme (ELP3, KAT7), 2 belong to acetylation reader (SP140, KIAA2026), and the other 6 belong to histone deacetylation enzyme (HDAC1, HDAC2, HDAC4, HDAC10, HDAC11, SIRT5). KAT7 (HBO1) belongs to the MYST superfamily and contains a specific region composed of acetyl-CoA binding motif and zinc finger(MYST domain). ELP3 belongs to the GNAT superfamily and has a conserved GNAT domain, and can acetylate lysine residues on histone H3<sup>[36]</sup>; HDAC1 and HDAC2 are Class I HDAC, which are nuclear proteins. HDAC4 and HDAC10 are Class II HDAC, which travel between cytoplasm and nucleus. HDAC11 is a Class IV HDAC with shared properties of Class I and CLASS II. It's an NAD + dependent enzyme. Figure 3A shows that among the 6 HDACs, only SIRT5 is a benign prognostic factor, while the rest are associated with poor prognosis of ovarian cancer.

In previous studies, these HAM proteins have been demonstrated to be closely associated with the progress of many cancer, and different types of HAMs have different effects on cancer. The catalytic subunit of the histone acetyltransferase KAT7

complex mediates the acetylation of histone H3K14ac, H4K5ac, H4K8ac and H4K12ac, thus playing a regulatory role in gene transcription, protein ubiquitination, and immune regulation<sup>[39, 40]</sup>.Studies have shown that KAT7 enhances the mechanical transduction pathway and membrane elasticity of ovarian cancer cells through the overexpression of preferential acetylation histone H4 of co-mediator JADE2, thus improving the migration ability and invasiveness of ovarian cancer cells<sup>[41, 42]</sup>.SP140 acts as an acetylation reader, preferentially occupying promoters of silenced genes with histone modification of H3K27me3, and is critical for transcriptional programs that support the macrophage state<sup>[43]</sup>.Histone deacetylases HDACs are the most important component of the gene labels we have established.Among them,HDAC1 and HDAC2 are class I HDACs, whose increased expression is an independent risk factor for poor prognosis of malignant ovarian tumors<sup>[44, 45]</sup>.In ovarian cancer, HDAC1 promotes cancer cell proliferation by increasing cyclin A<sup>[46]</sup>,and HDAC2 interferes with cisplatin-induced activation of DNA damage responses by remodeling chromatin<sup>[47]</sup>.In addition, many studies have shown that HDAC1 is also a good diagnostic or prognostic signature for lung cancer, gastric cancer, glioma, breast cancer and other cancers<sup>[47]</sup>.HDAC4 and HDAC10 are Class II HDAC, which are associated with proliferation, migration and invasion of a variety of cancers<sup>[47]</sup>.SIRT5 is a Class III HDAC that is involved in oxidative stress or metabolic homeostasis related to aging, degeneration or cancer<sup>[54]</sup>.Relevant studies revealed that SIRT5 can promote autophagy of gastric cancer cells, and SIRT5 can inhibit peroxisome-induced oxidative stress, thus protecting the liver and inhibiting the development of hepatoma cells<sup>[55, 56]</sup>.HDAC11 is the most recently discovered and smallest member of HDAC enzyme.At present, HDAC11 has been found to be associated with poor prognosis in liver, lung, ovarian, glioma, uveal melanoma and other cancers<sup>[57-59]</sup>.The results of the current literature are consistent with our findings, further confirming the widespread role of HAMs in cancer and supporting the prognostic value of these genes for ovarian cancer and other cancers.

452 We focused on the relationship between the risk signature and the tumor  
453 microenvironment. According to CIBERSORT algorithm analysis, 19 of the 22 types  
454 of immune cells had significant differences between tumor samples and normal  
455 samples. Among them, the expression of macrophages M0, macrophages M1, Tregs  
456 and CD4+ T cells were significantly up-regulated in cancer, indicating that they are  
457 important factors involved in ovarian cancer immunity. However, the infiltration of B  
458 cells and macrophage M1 showed significant differences between the samples in the  
459 high and low risk groups. Further correlation analysis showed a negative correlation  
460 between the degree of B cell infiltration and the risk score, suggesting a potential  
461 tumor suppressive effect of enhanced B cell infiltration. Recent studies have also  
462 shown that tumor-infiltrating B cells have antitumor effects and can combine with  
463 CD4+T cells to enhance local immune responses<sup>[57-59]</sup>. Subsequently, we performed the  
464 ESTIMATE algorithm to assess the overall immune status of ovarian cancer patients.  
465 Among them, the tumor purity was higher in the high-risk group, while the immune  
466 score was significantly reduced. This indicates that patients with high risk score had a  
467 poor level of tumor immune infiltration and were less able to kill tumor cells.  
468 Meanwhile, the result revealed a negative correlation between the risk score of the  
469 model and the level of immune infiltration.

470 In studies of immunotherapy efficacy, we examined the expression levels of  
471 several important immune checkpoint genes. We found that the expression of PD-L1,  
472 PD-L2, and CTLA4 was significantly increased in the low-risk group, and it can be  
473 speculated that the low-risk patients might have more obvious effects after receiving  
474 immune checkpoint inhibitors. Besides, TIDE algorithm revealed a higher likelihood  
475 of immune escape or immune dysfunction in high-risk patients, heralding poor  
476 response to immunoblocking therapy (ICB) in these patients. In addition, mutation  
477 analysis showed a significant decrease in TMB in the high-risk group compared to the  
478 low-risk group, and this difference may have an impact on patient survival. To further

479 verify the effect of receiving immunotherapy, we introduced IMVIgor210 cohort  
480 treated with PD-1/PD-L1 inhibitors as validation set. Results indicated that patients in  
481 the high-risk group had a lower, but not significant rate of objective response  
482 rate(CR/PR) after ICI treatment.Patients tended to perform differently on immune  
483 phenotype according to different risk scores. However, with the increase of risk score,  
484 the immune phenotype of patients vary from “inflamed”, to “exclude” and then to  
485 “desert”, indicating a decline in the level of immune infiltration and the effect of  
486 receiving immunotherapy<sup>[61]</sup>.

487 Our study also provides potential drugs for target therapy. We have identified 20  
488 compounds targeting HAMs-related signature using cMAP as potential target drugs for  
489 OC patients. These drugs include HDAC inhibitor, CDK inhibitor, PLK inhibitor,  
490 mTOR inhibitor, MEK inhibitor, etc. Of concern, 6 of the 20 small molecule  
491 compounds we identified act as histone deacetylation inhibitors (HDACI). HDACI is  
492 a diverse group of compounds that vary in structure, bioactivity and specificity. By  
493 affecting transcription, HDACI can halt the cell cycle, inhibit DNA repair, induce  
494 apoptosis and acetylation of non-histones, leading to downstream changes in gene  
495 expression<sup>[62]</sup>.On the one hand, dysregulation of transcription due to altered histone  
496 acetylation patterns is a mechanism for cancer occurrence, which is currently targeted  
497 by HDAC inhibitors;On the other hand, the traditional treatment for ovarian cancer is  
498 generally platinum-based therapy, while the high expression of HDAC family  
499 members increases the resistance of patients to platinum chemotherapy.Islam MM et  
500 al<sup>[63]</sup> have identified HDAC10 inhibitors as potential therapeutic targets for ovarian  
501 cancer, enhancing the efficacy of platinum drugs in malignant ovarian tumors.Besides,  
502 studies have shown that silencing HDAC1 by siRNA targeting leads to the induction  
503 of xenograft tumors that are sensitive to cisplatin therapy and can reduce drug  
504 resistance, which may be an effective strategy to improve the efficacy of cisplatin  
505 therapy<sup>[65]</sup>.In the sample analysis of GSE30161, our results verified this: high-risk  
506 patients had a significantly lower CR ratio compared with low-risk patients due to

507 higher HDAC expression. Therefore, the combination of HDAC inhibitors and  
508 platinum drugs may become one of the effective strategies for the treatment of ovarian  
509 cancer.

510 In this study, multiple data sets were included in the model construction and  
511 validation process to improve its accuracy, and the practical application ability of the  
512 model was studied from the perspectives of immunity, prognosis and treatment.  
513 However, there are some limitations to the study that need to be addressed. For  
514 example, the lack of information on clinical characteristics of patients with ovarian  
515 cancer, such as TNM classification, limits our ability to include clinical characteristics  
516 in risk assessment; Secondly, due to the relatively small amount of data on ovarian  
517 cancer patients and the differences in data processing between the data sets, it is  
518 difficult to validate the model with broader data. The robustness of the risk scoring  
519 model needs to be further evaluated in more cohorts. Additionally, our findings  
520 require long-term in vivo and in vitro experiments to further study and verify the  
521 specific mechanisms by which acetylation modulators influence cancer development.  
522 More details about the effect of histone regulation on cancer remain to be explored.

523 In conclusion, based on Cox regression analysis of expression profile of OC  
524 patients, we constructed a prognostic signature of ovarian cancer related to histone  
525 acetylation modulator genes, which can provide valuable reference for identifying  
526 high-risk groups of ovarian cancer and guidance for prognostic analysis of ovarian  
527 cancer patients. Subsequently, we completed the analysis of immune infiltration,  
528 immune therapy, and mutation profiles in high and low risk populations. Finally, our  
529 findings may help identify more effective targeted drugs and treatment strategies for  
530 ovarian cancer patients.

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676 **6. List of abbreviations**

677 **Ethical Approval**

678 Not applicable.

679 **Consent for publication**

680 Not applicable

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682 None

683 **Author Contributions**

684 Yujie Fang, Hecheng Wang, and Yanshuo Han conceived, designed, performed  
685 the study, and drafted the manuscript; Yujie Fang, Hao Zhang, Xu Guo, Jing Zhao,  
686 and Yanshuo Han analyzed the data; Yujie Fang, Hecheng Wang, Xiaoxu Zhang, and  
687 Yanshuo Han revised the manuscript. All authors read and approved the final  
688 manuscript.

689

690 **Data Availability Statement**

691 The datasets used or analyzed during the current study are available from the  
692 corresponding author on request.

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698

699 **Figure Captions**

700 **Tables**

701 **Table 1.**Scores and mechanisms of 20 potential drugs

702 **Supplementary Table 1.**List of 77 histone acetylation genes

703 **Supplementary Table 2.**Differential expression results of 77 HAMs.

704 **Supplementary Table 3.**Results of GSEA in GO

705 **Supplementary Table 4.**Results of GSEA in KEGG

706 **Supplementary Table 5.**Survival time and risk grouping of patient in TCGA.

707 **Supplementary Table 6.**Summary of previous literature on ovarian cancer gene

708 models.

709 **Supplementary Table 7.**The RiskScore and high- and low- group assignment of

710 samples from IMvigor210 cohort.

711 **Supplementary Table 8.**The RiskScore and high- and low- group assignment of  
712 samples from GSE30161 cohort.

713 **Supplementary Table 9.**Results of variation analysis between high and low risk

714 groups

715

716

717 **Figures**

718 **Figure 1.**The flow diagram of this study.

719       **Figure 2.**Differential expression analysis and Gene enrichment analysis.(A)Heat  
720 map of differentially expressed genes.(B)Volcano map of differentially expressed  
721 genes.(C)Enrichment analysis histogram of GO and KEGG analysis.(D)Enrichment  
722 analysis bubble plot of GO and KEGG analysis.(E,F)Enrichment analysis circle  
723 diagram of GO and KEGG analysis.

724       **Figure 3.**The construction of HAMs signature by LASSO regression. (A,B)  
725 Prediction of immune cell proportion in TCGA and GTEx sample.(C)Calibration  
726 curve of 3-Year OS.(D)Time-dependent ROC curves predicted 1,3,5-year prognostic  
727 performance in training cohort.(E)Kaplan - Meier curves to compare overall survival  
728 of high - risk and low - risk groups in training cohort.

729       **Figure 4.**Validation of HAMs signature in GEO cohort. (A) Kaplan - Meier  
730 curves to compare overall survival of high - risk and low - risk groups in validation  
731 cohort.(B)Time-dependent ROC curves predicted 1,3,5-year prognostic performance  
732 in validation cohort.(C)Risk score distribution.(D)Individual status of survival.(E)A  
733 heat map of the differentially expressed genes between high and low risk

734       **Figure 5.**The relationship between the HAMs signature and immune infiltration.  
735 (A,B) Prediction of immune cell proportion in TCGA and GTEx  
736 sample.(C)Comparison of relative immune cell abundance in high-risk and low-risk  
737 groups.(D)correlation of Risk score and immune infiltrates.(E)Comparison of tumor  
738 immune score in high-risk and low-risk groups based on ESTIMATE R package.\*:

739 Statistically significant p<0.05; \*\*: Statistically significant p < 0.01.

740       **Figure 6.**Evaluation of immune status between low-risk and high-risk  
741 groups.(A)Expression level of immune checkpoint genes.(B)Tumor immune  
742 dysfunction and exclusion scores in high and low risk group.(C)Relationship between  
743 risk score and immune phenotype in IMVIgor210 cohort.(D)Objective response rates  
744 in the low-risk group (ORR=CR+PR).

745       **Figure 7.**Mutation profile and HAMs risk groups.(A,B)Mutation profiles of high  
746 and low risk groups.(C)TMB differences between high and low risk groups.(D)The  
747 correlation between TMB and survival

748       **Figure 8.**Targeted therapy analysis.(A)Efficacy of platinum/paclitaxel in the high  
749 and low risk groups.(B)Relationship between therapeutic effect and  
750 survival(C)Potential targeted drugs predicted by cMAP analysis.(the abscissa of the  
751 heatmap is the compound and score, the ordinate is the cell line, red indicates  
752 sensitivity to the compound, and blue indicates insensitivity to the compound)

753

754       **Figure 9.**(A)The KM survival curves revealed contrasting survival possibili  
755 ty predicted by varying expression of signature genes.(B)Protein expression of  
756 crucial HAMs in Ovary Cystadenocarcinoma Serous and normal ovary tissue  
757 based on the HPA database.(HDAC1 tumor:HPA029693,Staining:Medium,Quantit  
758 y:>75% ;HDAC1 normal:CAB068191,Staining:Medium,Quantity:>75%-25% ;HD  
759 AC10 tumor:CAB045977,Staining:Medium,Quantity:>75% ;HDAC10 normal:CAB

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760 045977,Staining:High,Quantity:>75% ;KAT7 tumor:HPA044470,Staining:Medium,  
761 Quantity:75%-25% ;KAT7 normal:HPA044470,Staining:Medium,Quantity:>75%-2  
762 5%)(C,D)Protein-protein interactive network of prognostic HAMs.(C)Protein-prot  
763 ein interaction network by STRING database.(D)Protein interaction analysis by  
764 geneMania database.

765 **Supplementary Figure 1.**The results of lasso regression analysis.

766 **Supplementary Figure 2.**Immune cell infiltration and clinical grading.

767 **Supplementary Figure 3.**Box plot of Tumor immune dysfunction and exclusion  
768 scores in high and low risk groups.

769 **Supplementary Figure 4.**KM survival curves of model genes.

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**Table 1:**

Name	Comprehensive Score	MOA(mechanism of action)
AS-703026	98.41	MEK inhibitor
ISOX	96.26	HDAC inhibitor
apicidin	96.09	HDAC inhibitor
MST-312	96.02	telomerase inhibitor
WT-171	94.31	HDAC inhibitor
cytochalasin-b	94.27	microtubule inhibitor, phagocytosis inhibitor
vorinostat	92.59	HDAC inhibitor, cell cycle inhibitor
epoxycholesterol	91.54	LXR agonist
WYE-354	91.21	mTOR inhibitor
SB-590885	91.06	RAF inhibitor
cytochalasin-d	89.01	actin polymerization inhibitor, actin stabilizer
THM-I-94	88.99	HDAC inhibitor, apoptosis stimulant, cell cycle inhibitor
WYE-125132	88.82	mTOR inhibitor, PI3K inhibitor
trichostatin-a	87.17	HDAC inhibitor, CDK expression enhancer, ID1 expression inhibitor
wortmannin	86.89	PI3K inhibitor, ATM kinase inhibitor, PLK inhibitor,etc
torin-2	86.04	mTOR inhibitor
BI-2536	85.47	PLK inhibitor, apoptosis stimulant, cell cycle inhibitor, protein kinase inhibitor
fluticasone	85.25	glucocorticoid receptor agonist
WAY-170523	85.1	metalloproteinase inhibitor
fenpiverinium	85.05	acetylcholine receptor antagonist

Figure 1.JPEG

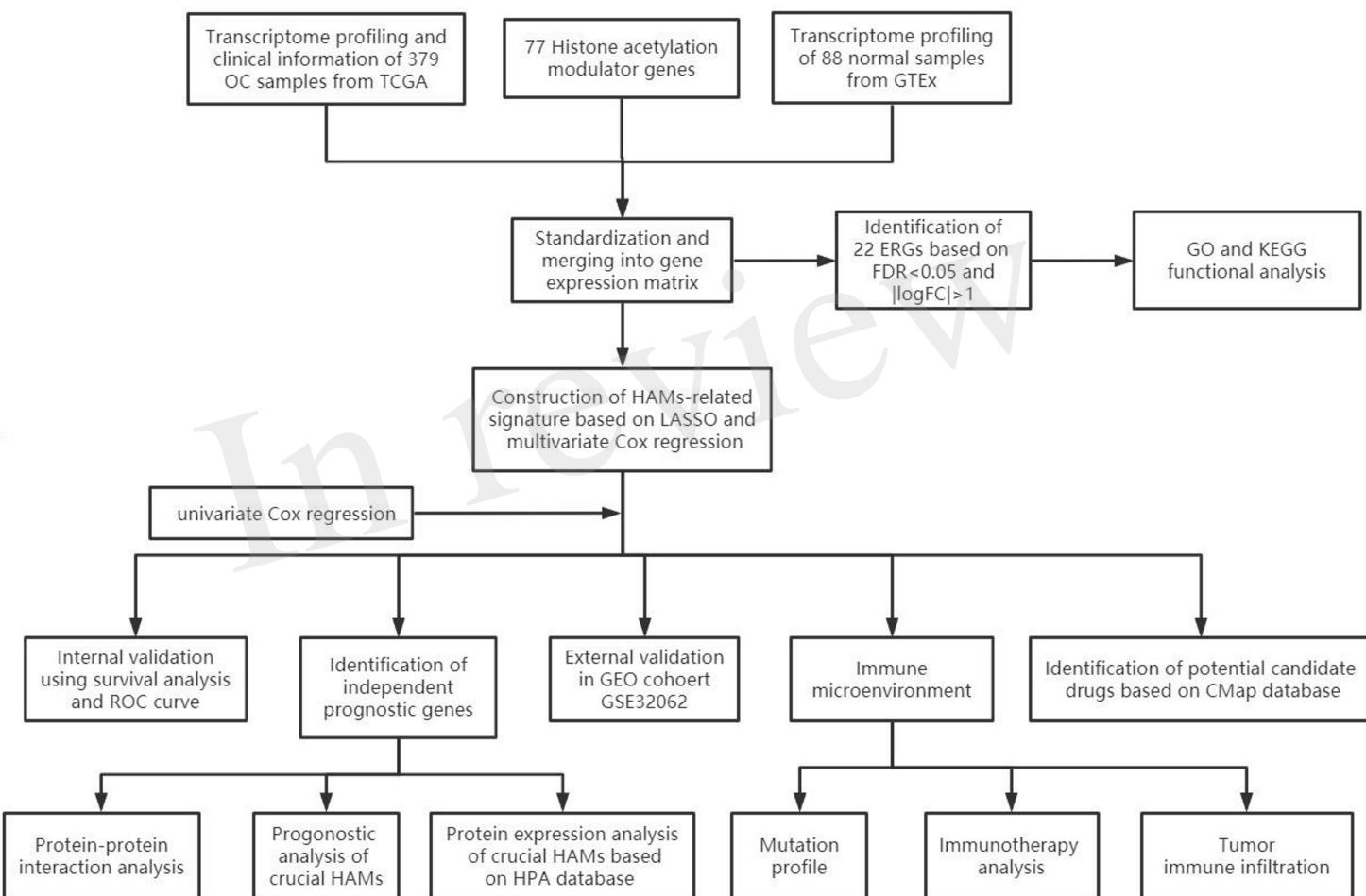


Figure 2.JPEG

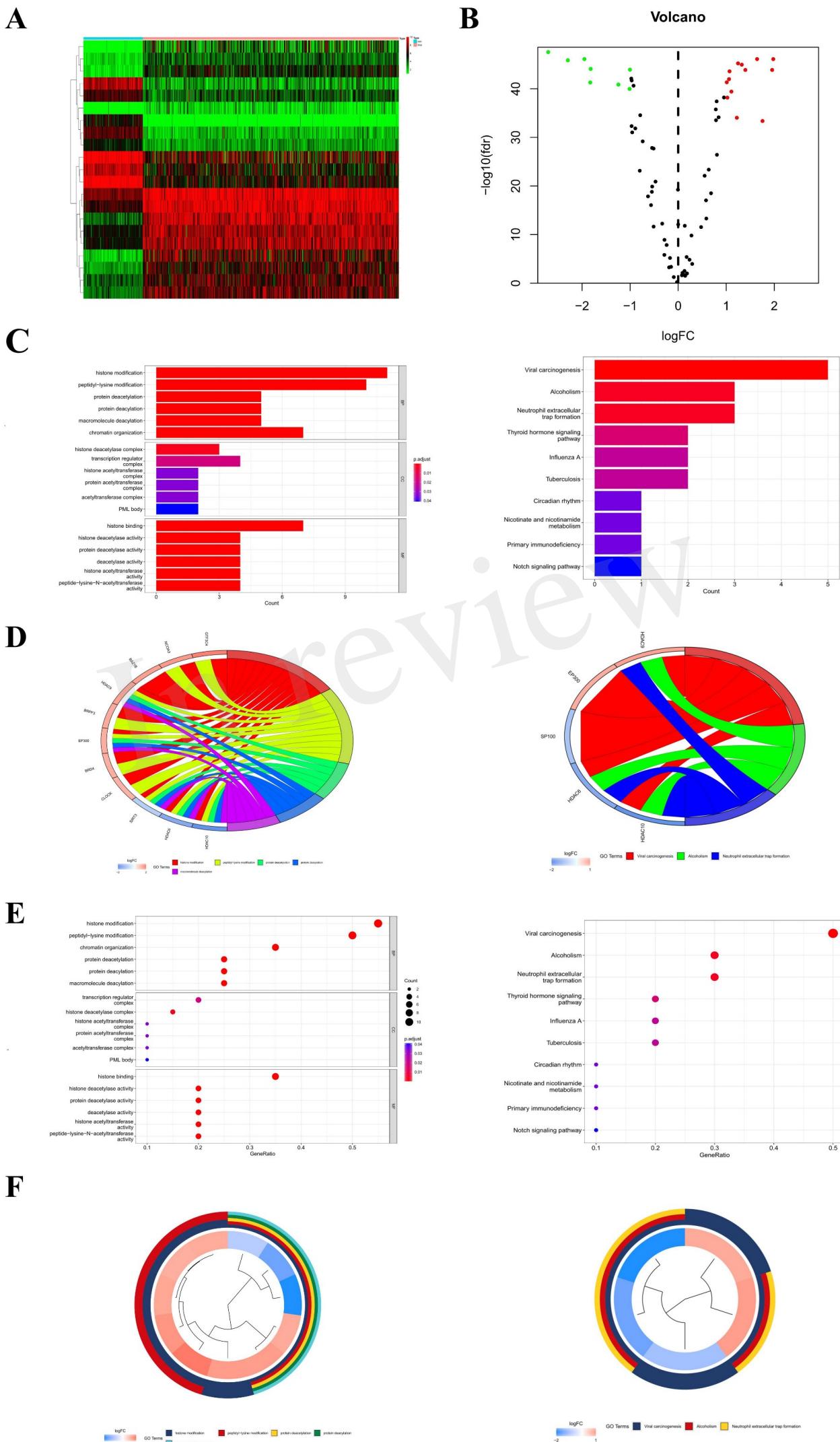
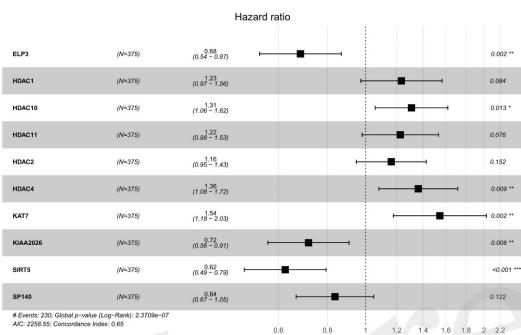
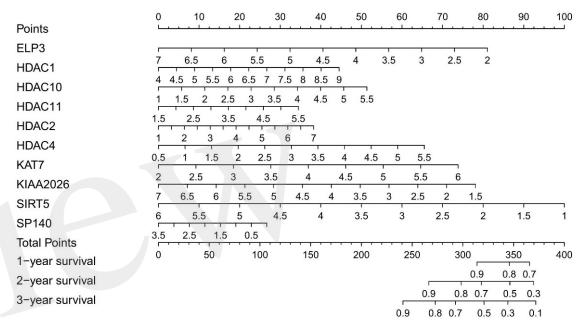


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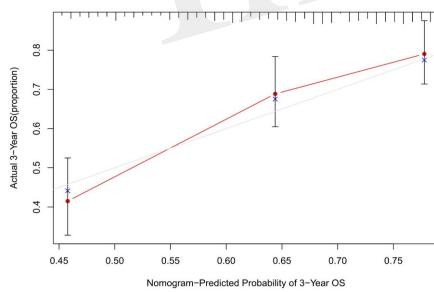
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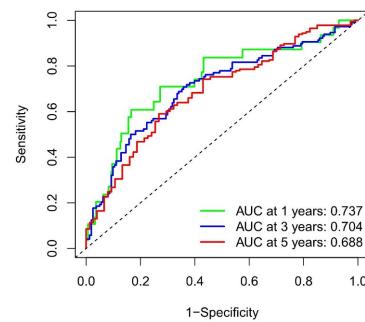
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C



D



E

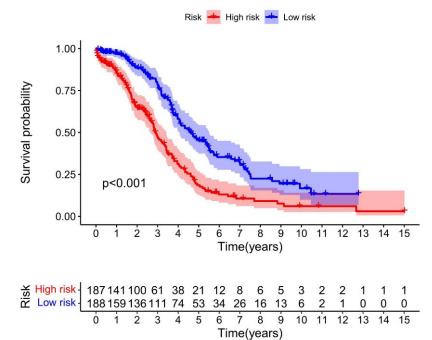


Figure 4.JPG

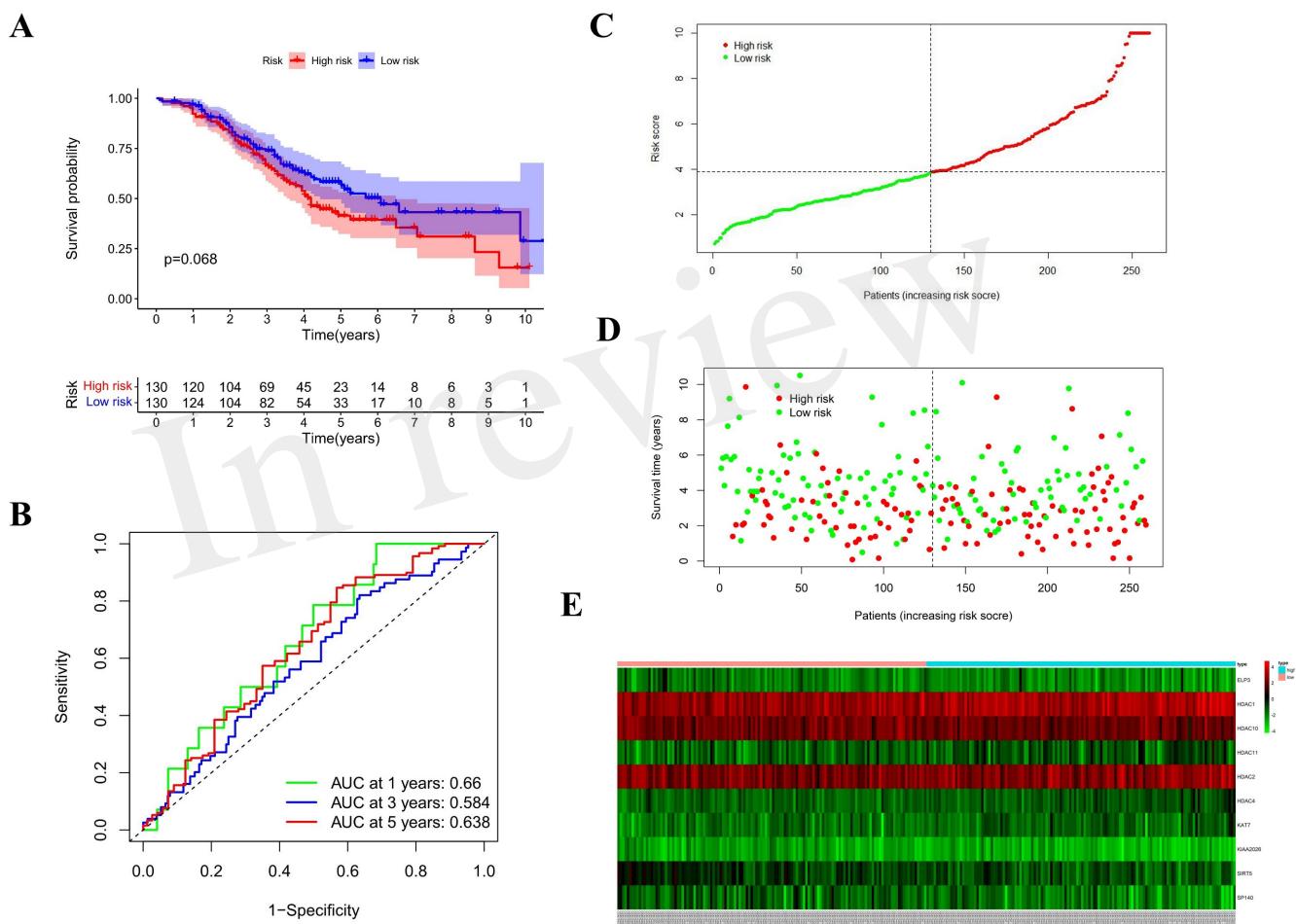


Figure 5.JPEG

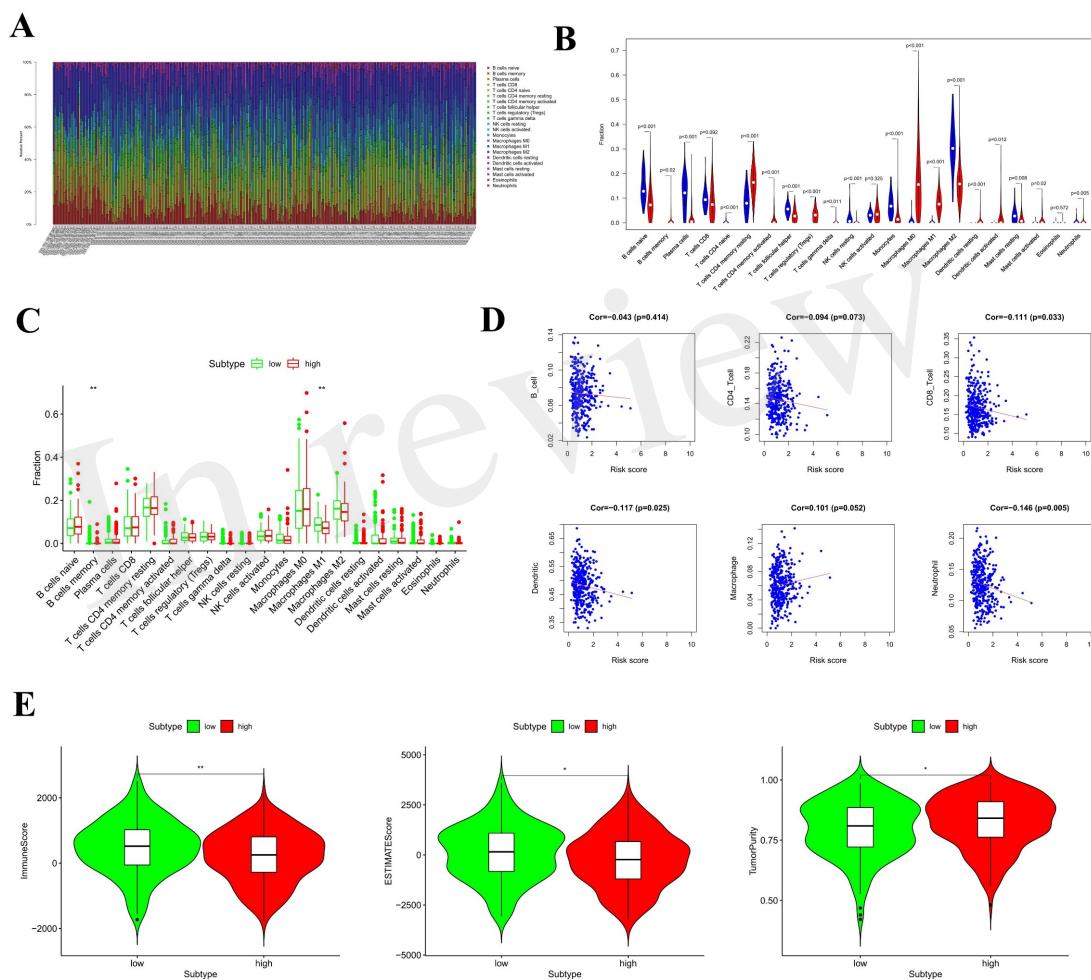


Figure 6.JPG

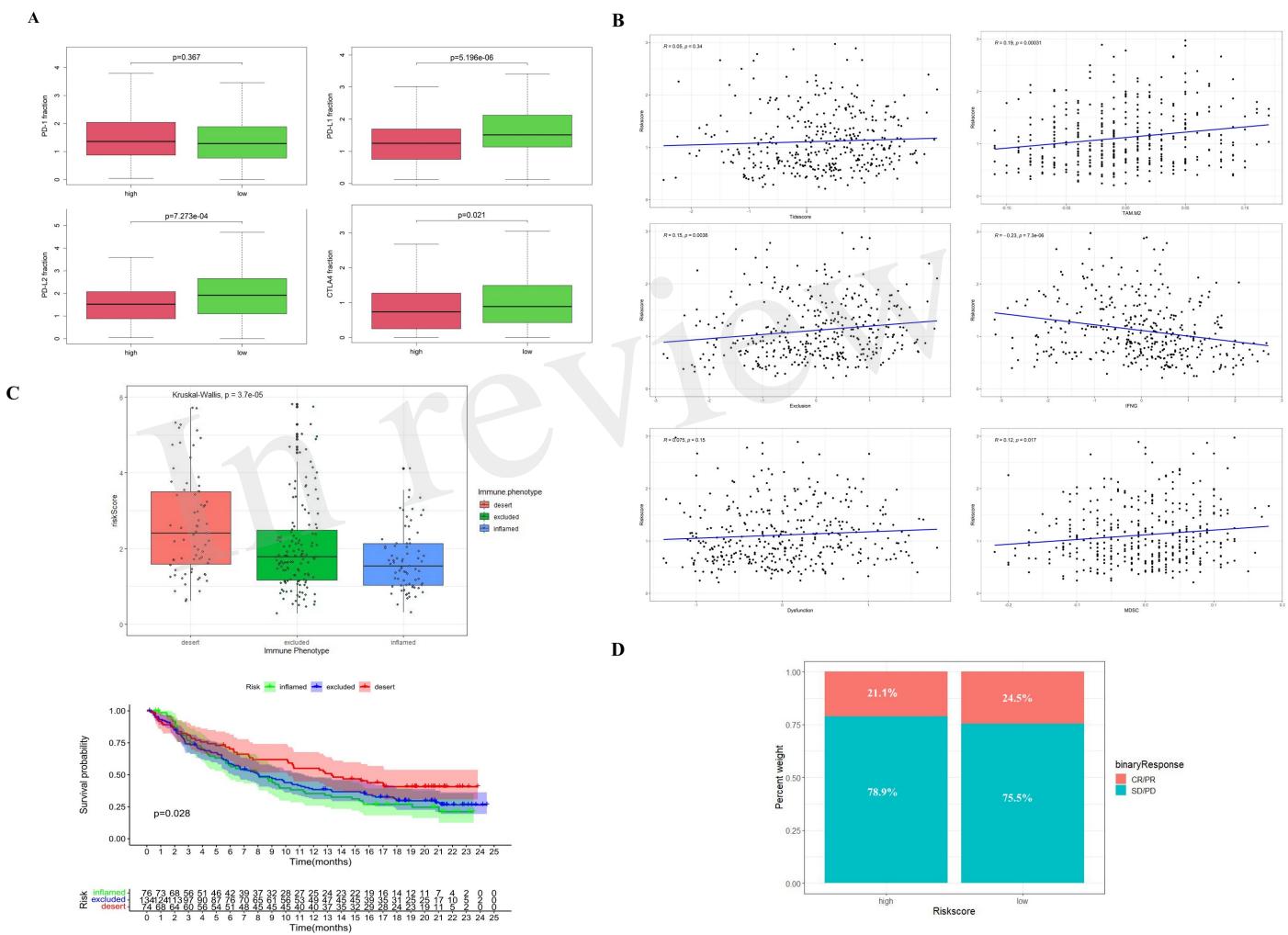
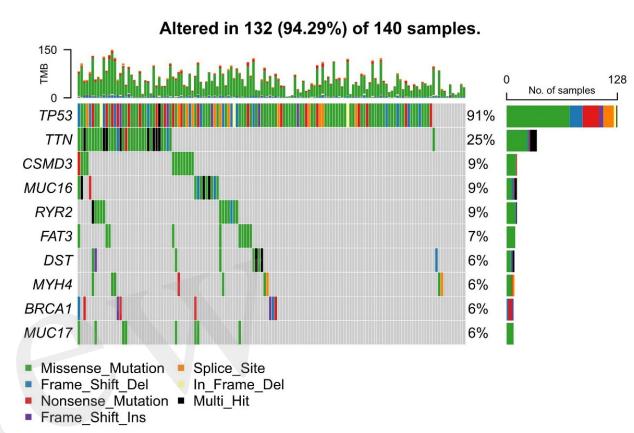


Figure 7.JPG

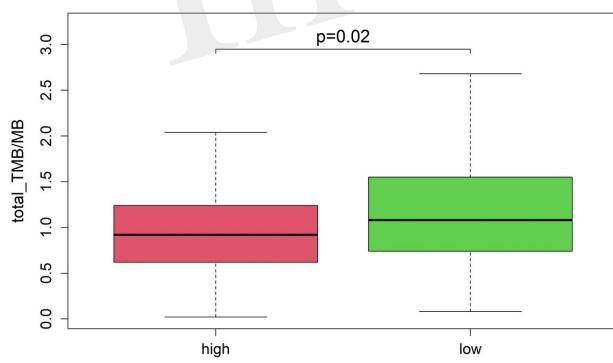
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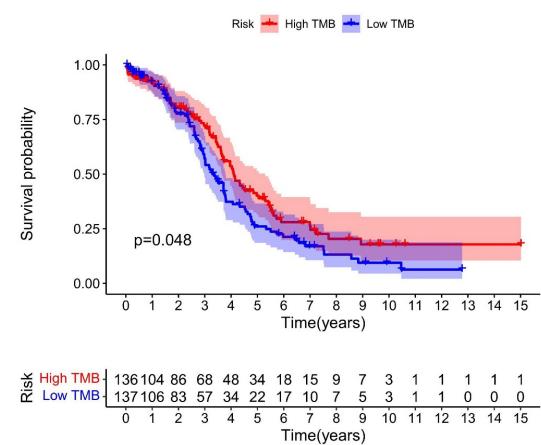
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C

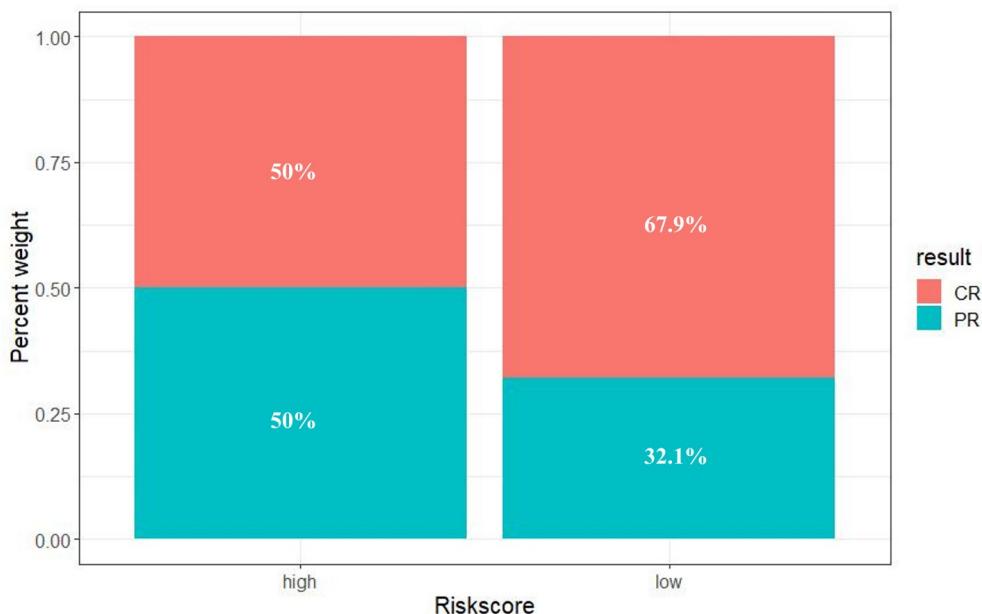


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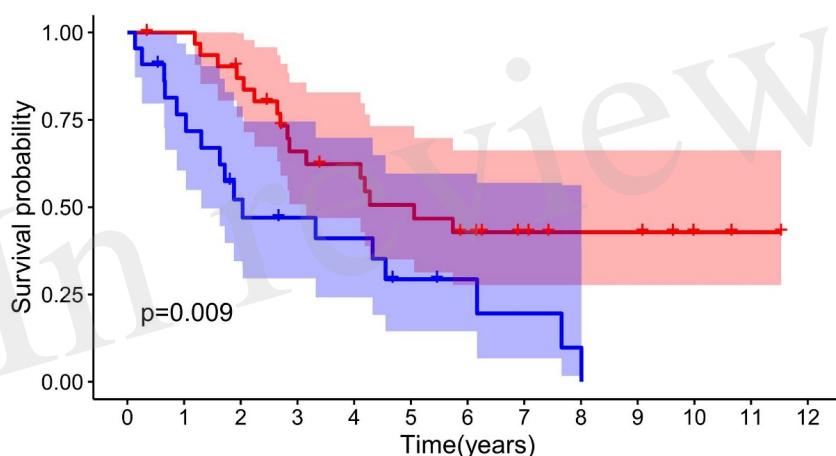


**A**

Figure 8.JPG

**B**

Risk PR CR



Risk	PR	CR																		
32	31	26	18	16	13	10	7	5	5	2	1	0	0	0	0	0	0	0	0	
0	1	2	3	4	5	6	7	8	9	10	11	12	0	1	2	3	4	5	6	

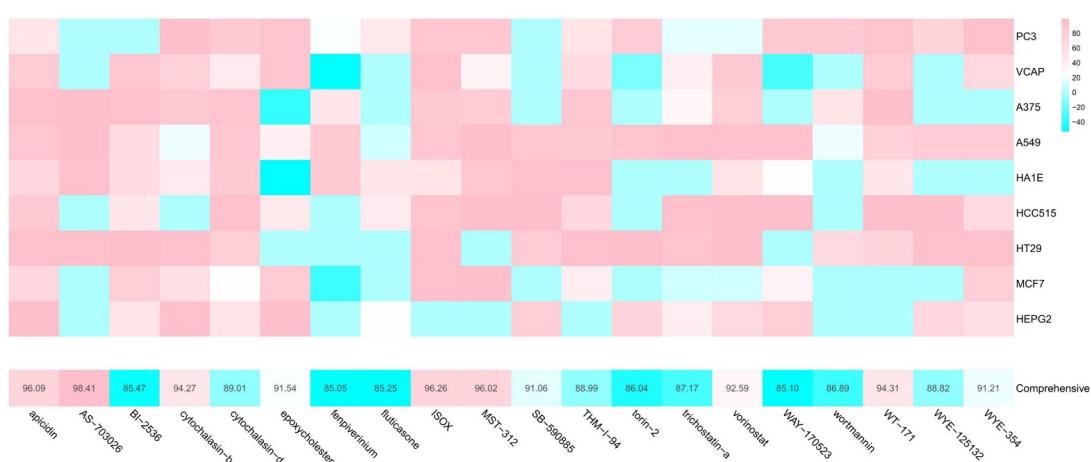
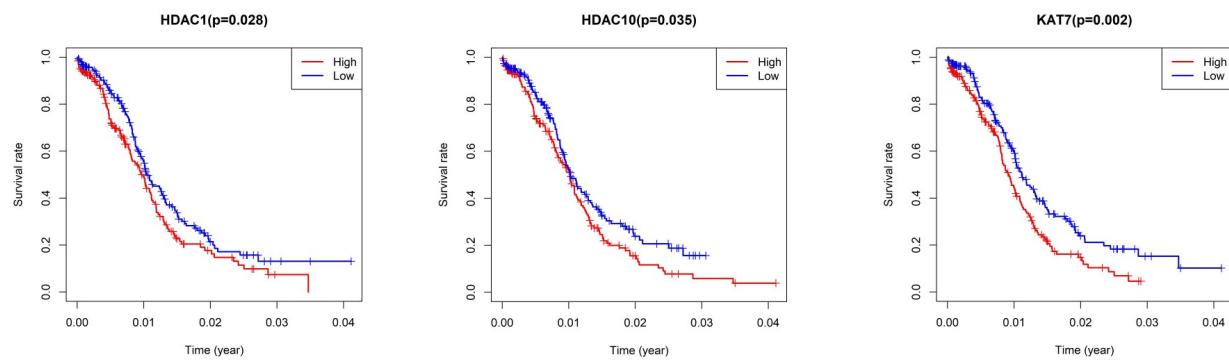
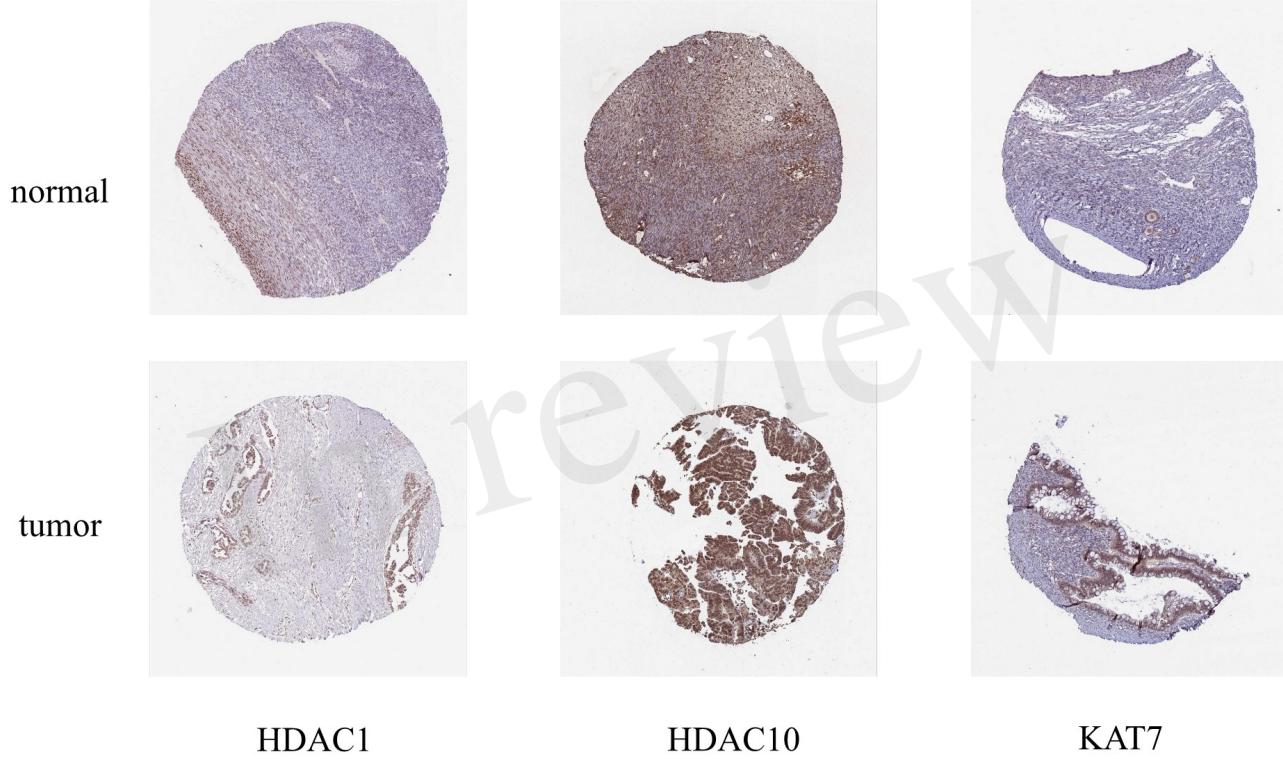
**C**

Figure 9.JPG

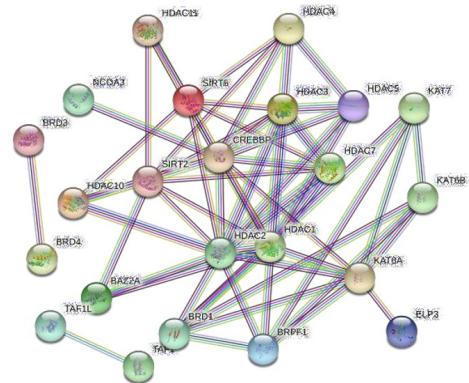
**A**



**B**



**C**



**D**

