

Supplementary Figures

Machine learning developed a macrophage signature for predicting prognosis, immune infiltration and immunotherapy features in head and neck squamous cell carcinoma

Authors: Yao Wang, BD^{1,2,3}, Ya-Kui Mou, MD^{1,2,3}, Wan-Chen Liu, BD^{1,2,3}, Han-Rui Wang, BD^{1,2,3}, Xiao-Yu Song, MD^{1,2,3}, Ting Yang^{1,2,3}, Chao Ren, MD, PhD^{1,2,3,4*}, Xi-Cheng Song, MD, PhD^{1,2,3*}

Yao Wang, Ya-Kui Mou and Wan-Chen Liu contributed equally to this work.

Affiliations: 1Department of Otorhinolaryngology, Head and Neck Surgery, Yantai Yuhuangding Hospital, Qingdao University, Yantai, China.

2Shandong Provincial Clinical Research Center for Otorhinolaryngologic Diseases, Yantai Yuhuangding Hospital, Yantai, China

3Yantai Key Laboratory of Otorhinolaryngologic Diseases, Yantai, China.

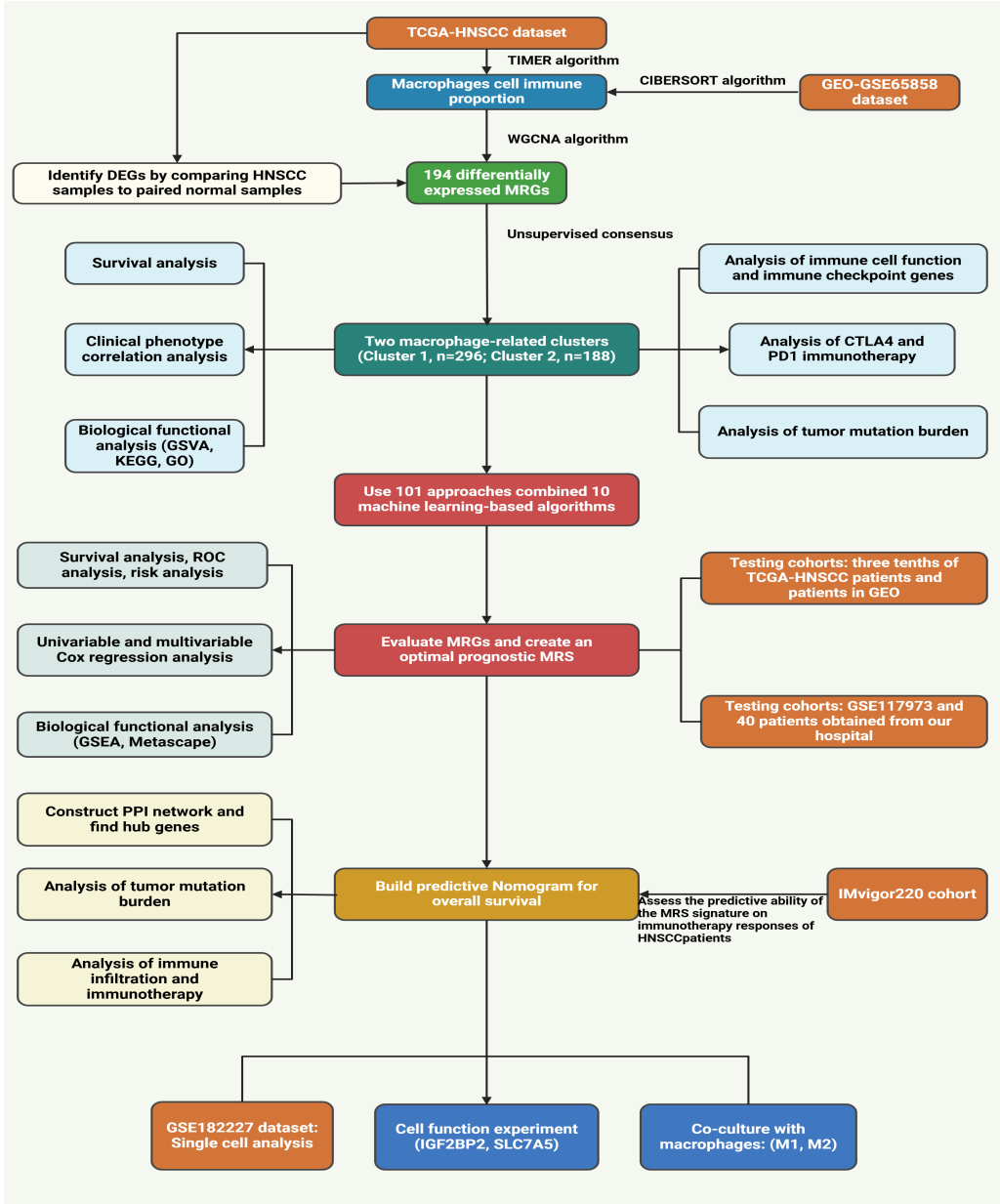
4Department of Neurology, Yantai Yuhuangding Hospital, Qingdao University, Yantai, China, 264000.

+Correspondence:

Dr. Chao Ren. Department of Otolaryngology, Head and Neck Surgery. Yantai Yuhuangding Hospital, Qingdao University, No.20, East Road, Zhifu District, Yantai 264000, China. Tel: +86535 6691999; Fax: +86535 6240341; E-mail: renchaotg@126.com.

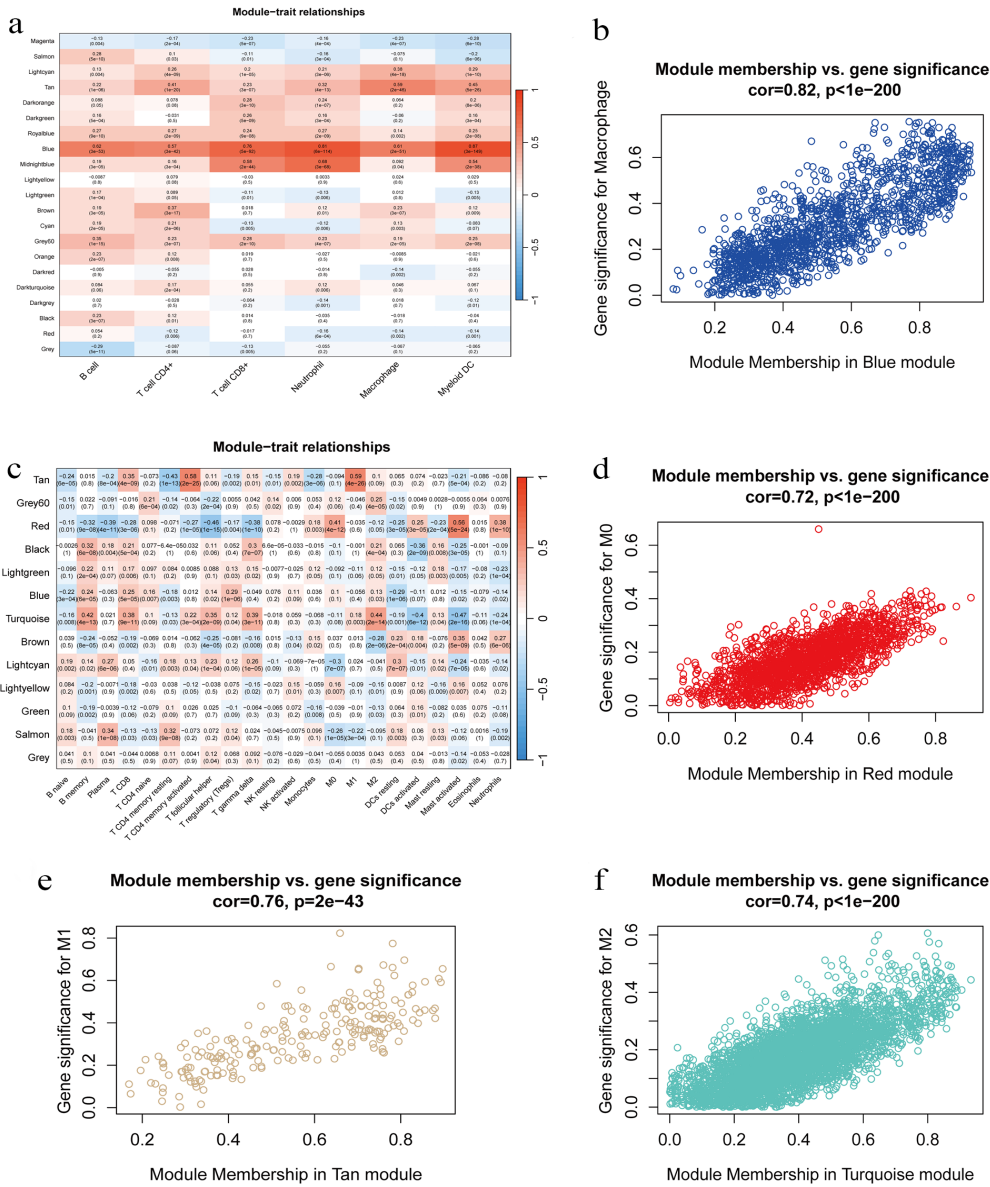
Prof. Dr. Xicheng Song. Department of Otolaryngology, Head and Neck Surgery. Yantai Yuhuangding Hospital, Qingdao University, No.20, East Road, Zhifu District, Yantai 264000, China. Tel: +86535 6691999; Fax: +86535 6240341; E-mail: drxchsong@163.com.

Supplementary Figure S1



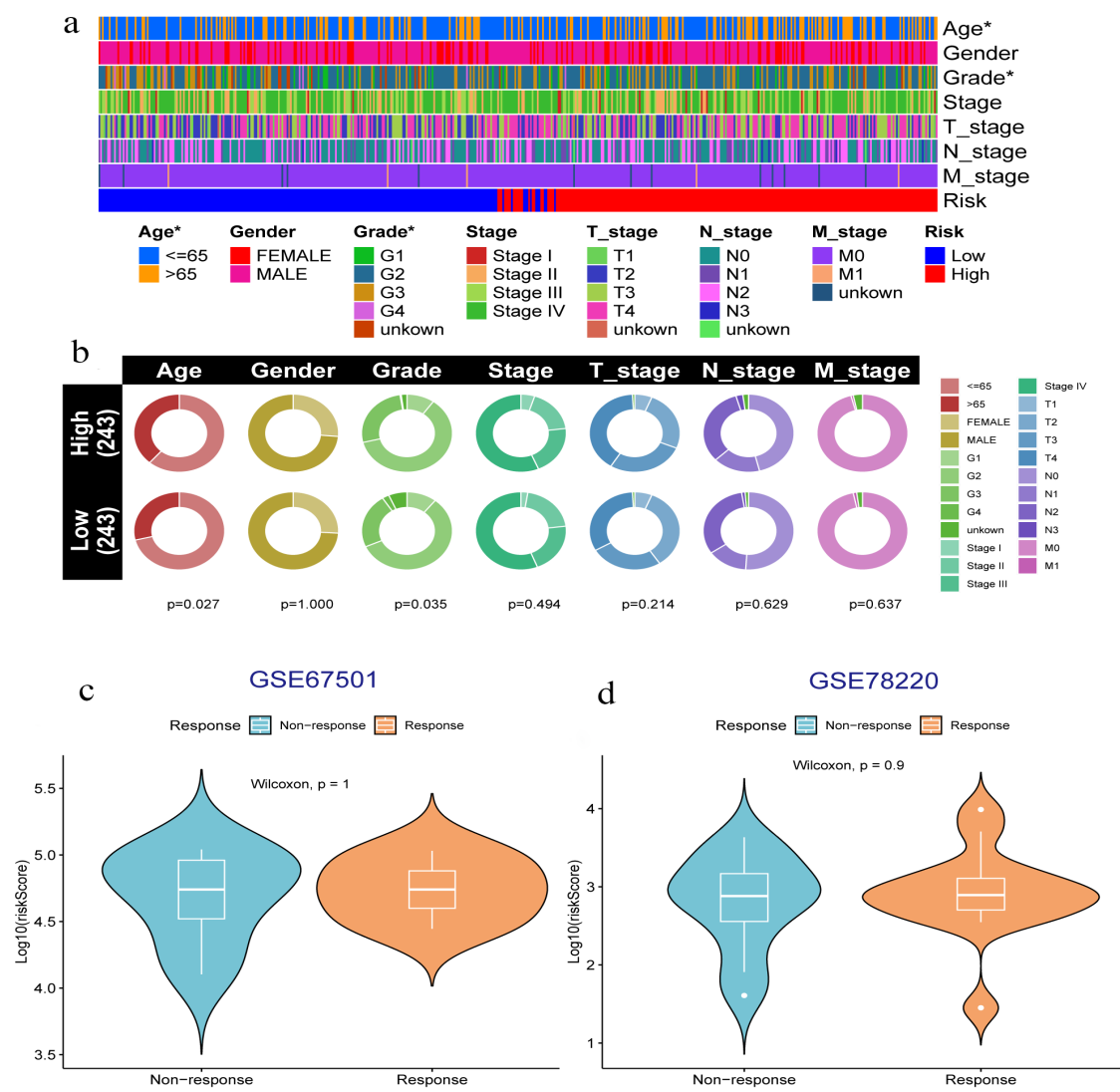
The flowchart of the design of this study.

Supplementary Figure S2



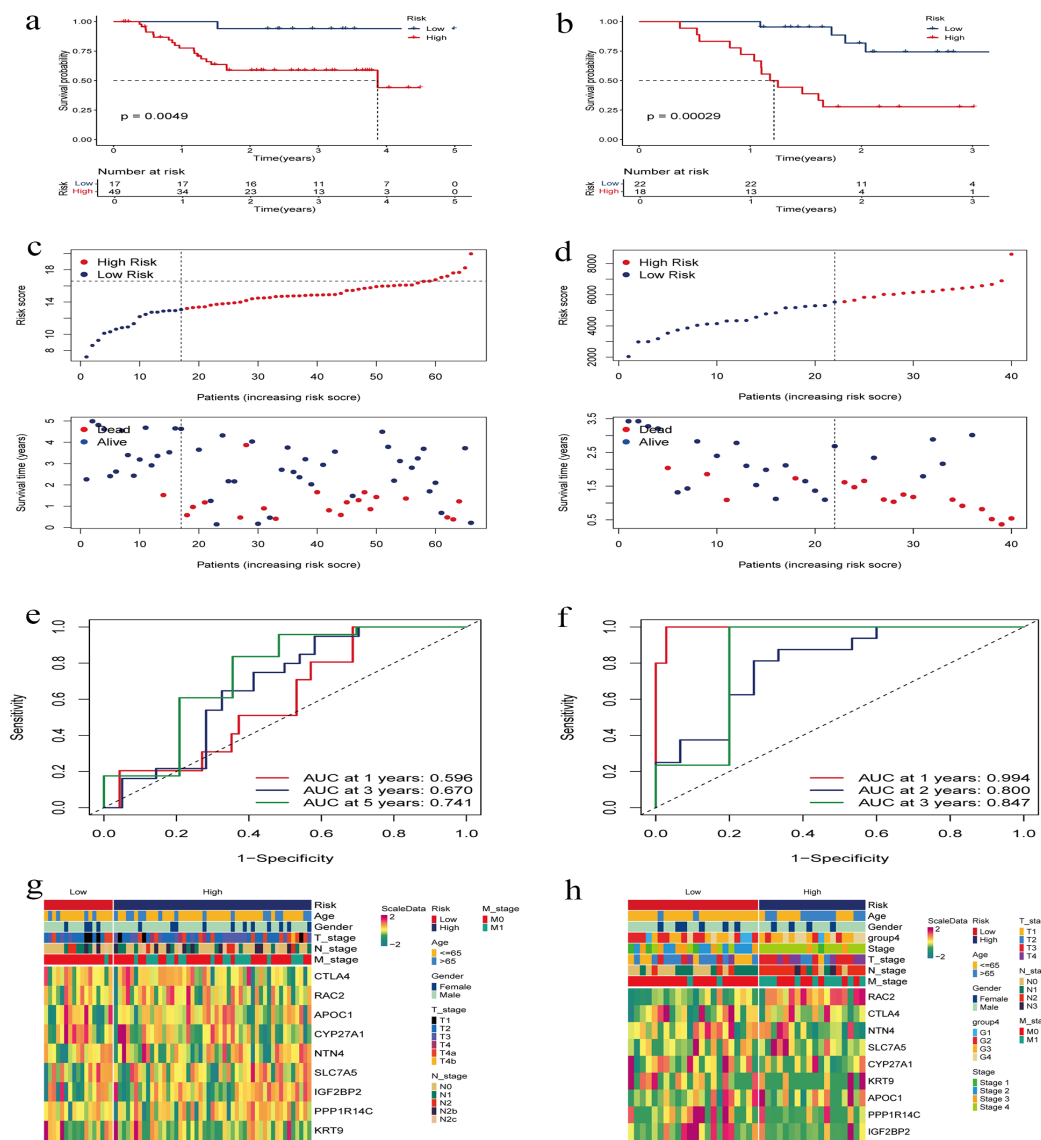
Spearman's rank correlation test was used to ascertain gene significance (GS) and module membership (MM), linking module eigengenes (MEs) with macrophages, implementing to augment the findings.

Supplementary Figure S3



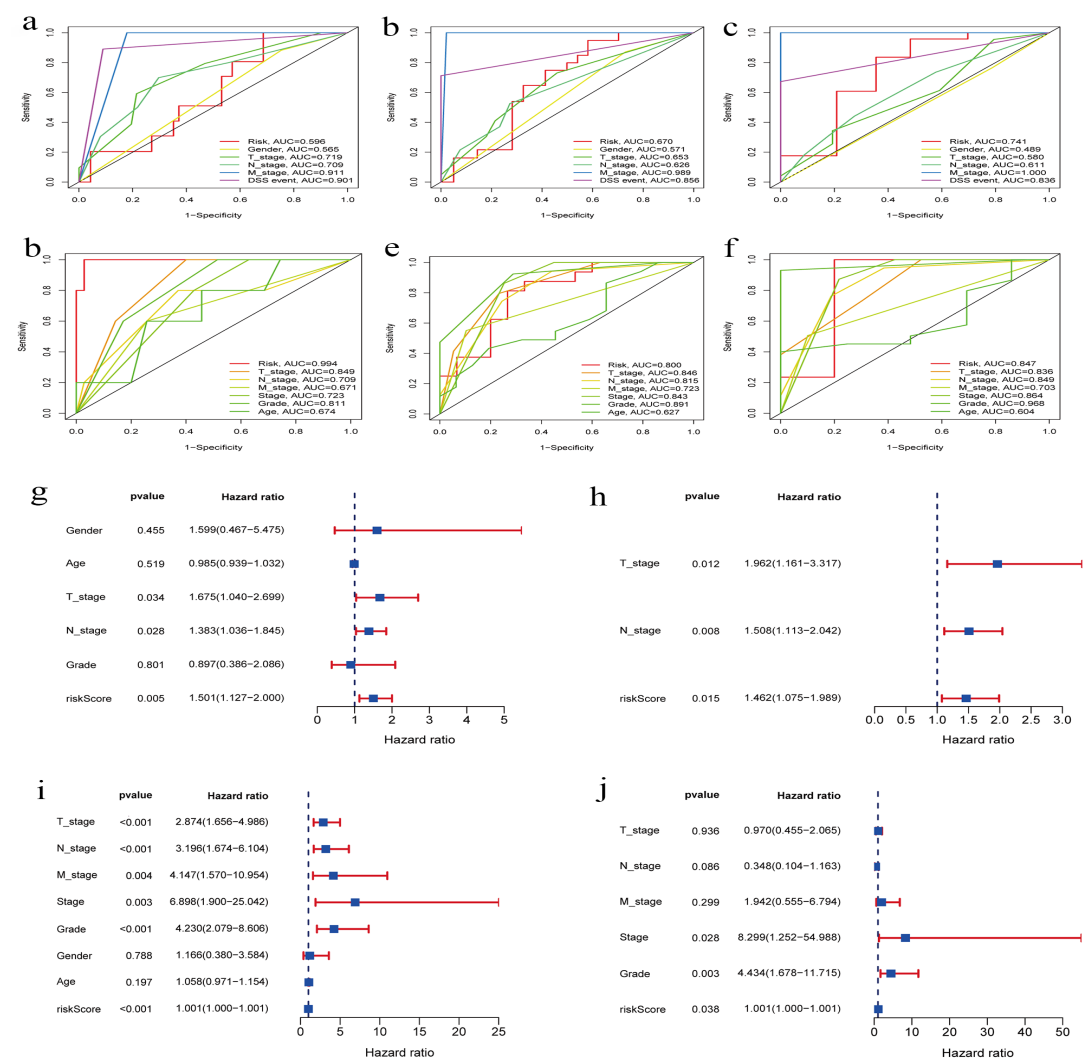
Correlation between MRS model and clinicopathological characteristics based on TCGA-HNSCC dataset. (a, b) The heatmap and pie graphs displayed the differences in age, gender, pathological grade, and clinical stage distribution among patients in varying risk groups. (c) The box plot compared the risk score between response and non-response groups underwent immunotherapy in GSE67501 dataset. (d) The box plot compared the risk score between response and non-response groups underwent immunotherapy in GSE78220 dataset.

Supplementary Figure S4



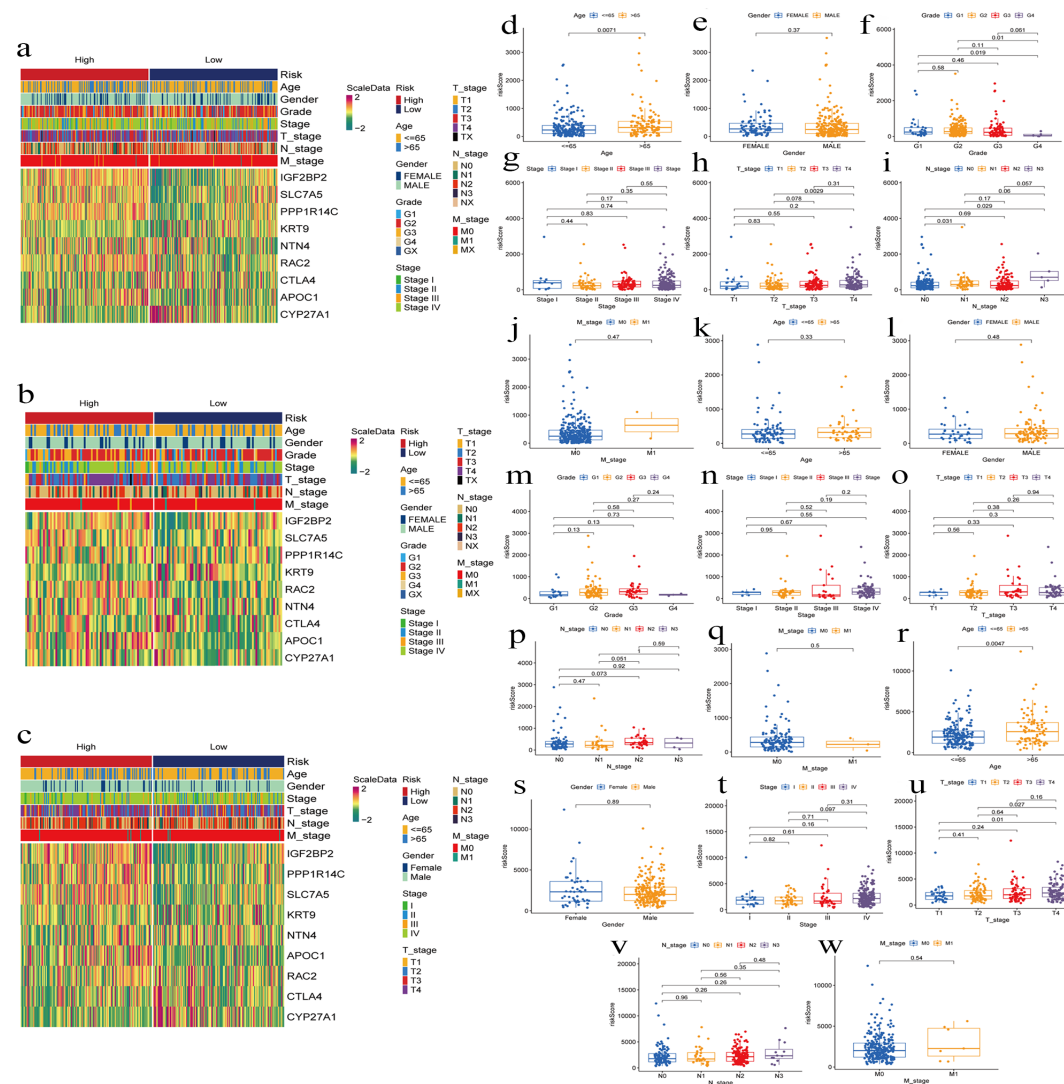
Survival analysis and ROC analysis for the two external cohorts. The Kaplan – Meier curve showed the survival analysis of HNSCC patients in GSE117973 (a), transcriptome data from 40 HNSCC patients (b). (c-d) The distribution of risk scores and survival statuses for patients with HNSCC in the two risk group in GSE117973 and transcriptome data from 40 HNSCC patients. (e-f) The ROC analysis showed the AUCs for 1-, 2-, 3-, and 5-year OS of patients with HNSCC in GSE117973 and transcriptome data from 40 HNSCC patients. (g-h) The heatmap displayed the correlation between genes in MRS and clinicopathological manifestation in GSE117973 and transcriptome data from 40 HNSCC patients.

Supplementary Figure S5



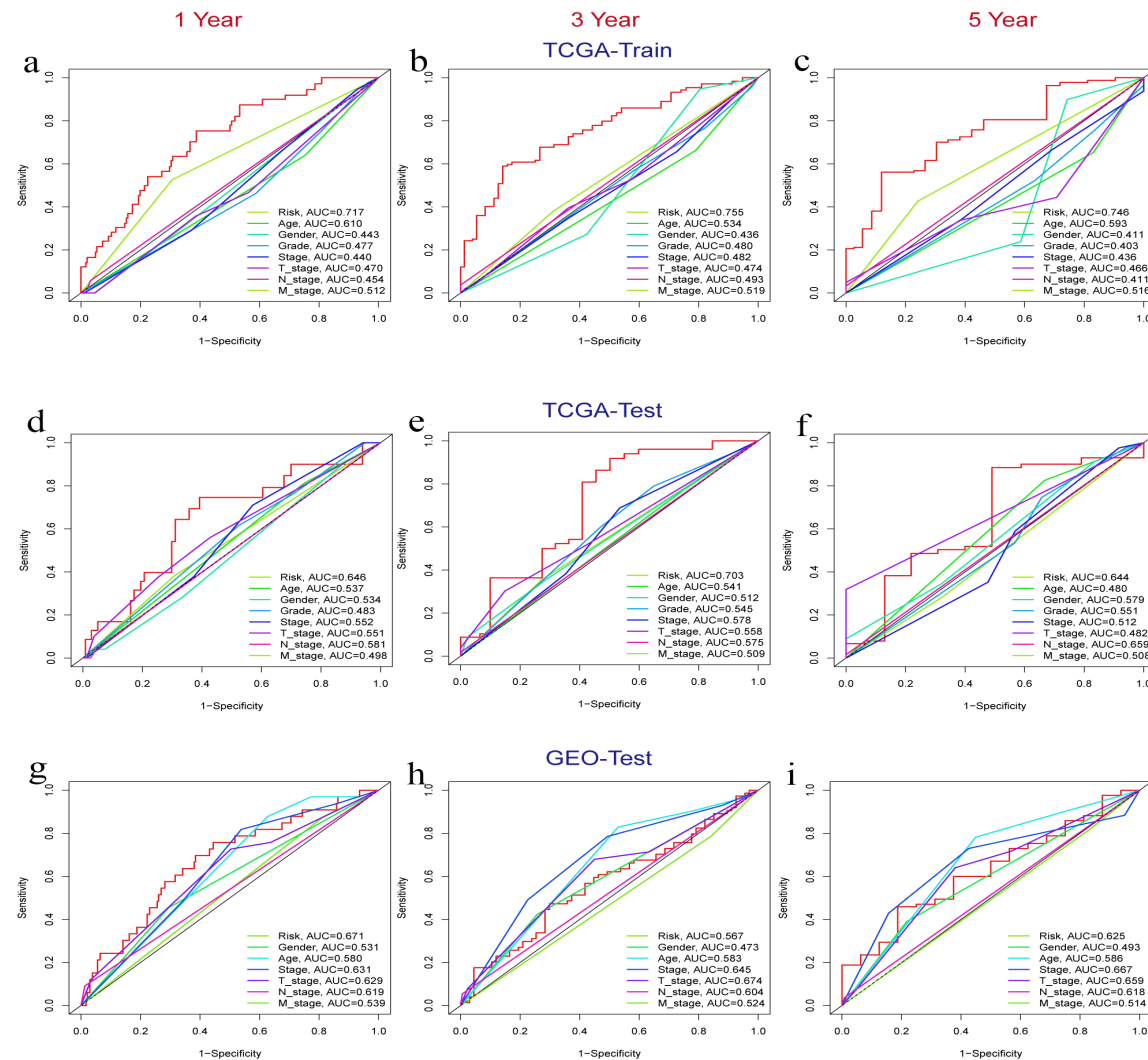
ROC curve analysis and Cox regression analysis of the two external datasets. (a-c) ROC analysis compared the predictive power of the MRS signature and other clinicopathological indicators in terms of 1-, 3-, and 5-year OS of patients with HNSCC in GSE117973. **(d-f)** ROC analysis compared the predictive power of the MRS signature and other clinicopathological indicators in terms of 1-, 2-, and 3-year OS of patients with HNSCC in transcriptome data from 40 HNSCC patients. **(g-h)** Univariate and multivariate Cox regression analyses of GSE117973 dataset. **(i-j)** Univariate and multivariate Cox regression analyses of dataset of transcriptome data from 40 HNSCC patients.

Supplementary Figure S6



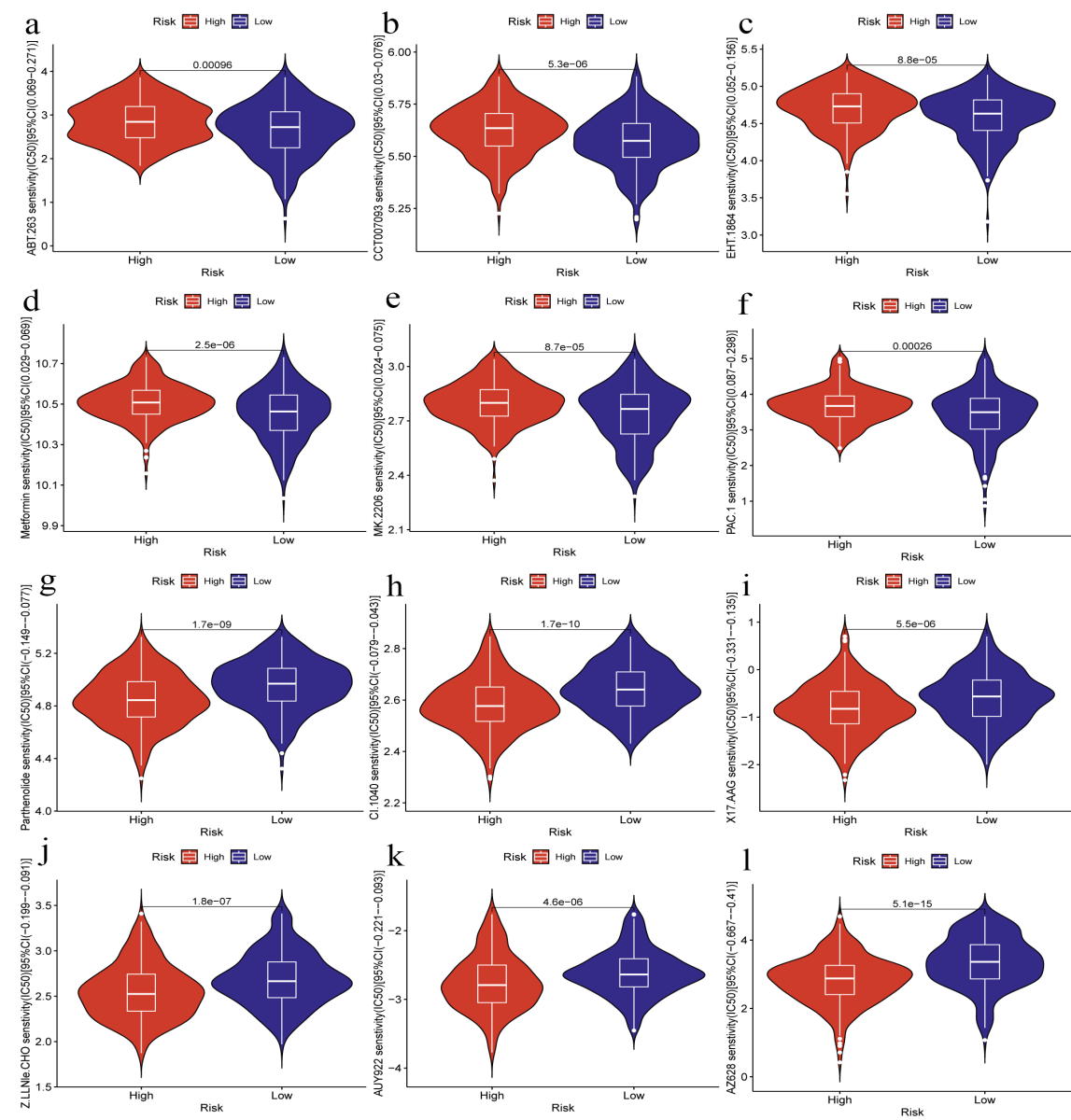
The MRS signature was associated with the clinicopathological characteristics of patients with HNSCC. (a-c) The heatmap displayed the correlation between genes in MRS and clinicopathological manifestation in TCGA training, TCGA testing and GSE65858 cohort, respectively. (d-j) Box plots showed the disparities of risk score in terms of age, gender, grade, pathological stage and TNM status in TCGA training cohort, separately. (k-q) Box plots showed the disparities of risk score in terms of age, gender, grade, pathological stage and TNM status in TCGA testing cohort, separately. (r-w) Box plots showed the disparities of risk score in terms of age, gender, grade, pathological stage and TNM status in GSE65858 cohort, separately. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Supplementary Figure S7



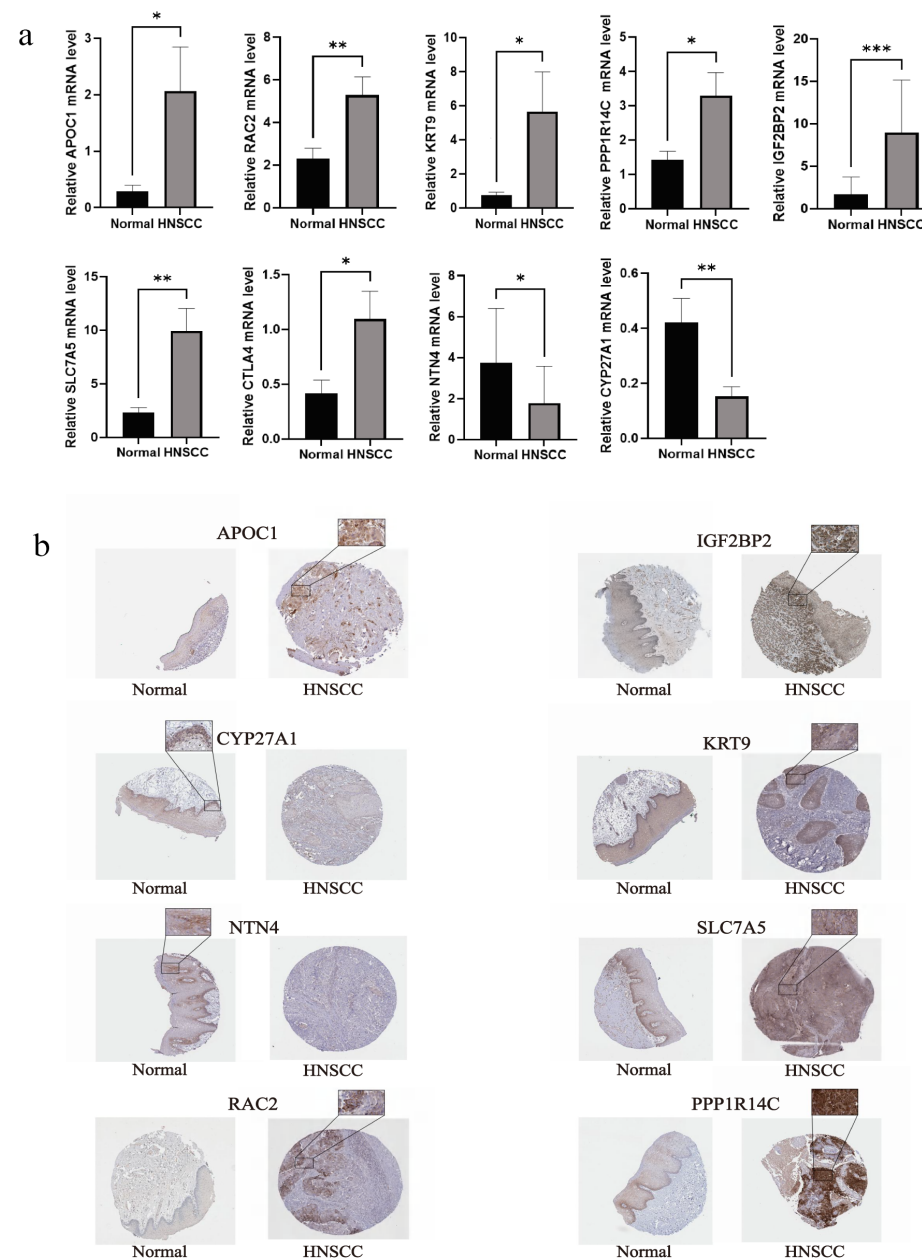
ROC curve analysis of the MRS model. (a-c) ROC analysis compared the predictive power of the MRS signature and other clinicopathological indicators in terms of 1-, 3-, and 5-year OS of patients with HNSCC in TCGA training cohort. **(d-f)** ROC analysis compared the predictive power of the MRS signature and other clinicopathological indicators in terms of 1-, 3-, and 5-year OS of patients with HNSCC in TCGA testing cohort. **(g-i)** ROC analysis compared the predictive power of the MRS signature and other clinicopathological indicators in terms of 1-, 3-, and 5-year OS of patients with HNSCC in GSE65858 cohort.

Supplementary Figure S8



(a-l) Chemotherapeutic drugs sensitivity analysis between high- and low-risk groups.

Supplementary Figure S9



Experimental verification of expression levels of nine genes in MRS. (a) The box plots compared the levels of mRNA expression of genes in MRS between cancer tissue and its neighbouring tissue using RT-qPCR. **(b)** The immunohistochemistry pictures obtained from the HPA database visualized the protein expression levels of genes in MRS between cancer and normal tissue. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.