# Major Depressive Disorder Trait Genes Promote Triple-Negative Breast Cancer Progression and Predict Immunotherapy Responses

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Research Article

**Additional Declarations:** No competing interests reported.

# Abstract

TNBC is the most malignant subtype of breast cancer and there are no accurate and effective therapeutic targets. Immunotherapy is a promising approach for the treatment of TNBC. Anxiety and depression are among the most common concomitant symptoms in BC.MDD affects the functioning of the immune system, and its immune-related genes not only influence the pathophysiology of MDD, but may also increase the risk of BC recurrence and metastasis.

This study revealed significant differences in T-lymphocyte infiltration between the high-risk and low-risk groups of TNBC differentiated on the basis of the characteristic inflammatory genes of MDD, which can help to screen the population for immunotherapy benefit and provide new ideas for future immunotherapy of TNBC. We aimed to identify MDD-related genes involved in the pathogenesis of TNBC and to provide predictive immunotherapy biomarkers for TNBC.

# 1. INTRODUCTION

Data from GLOBACAN confirms that breast cancer (BC) has surpassed lung cancer as the most prevalent malignancy globally, with approximately 2.26 million cases worldwide in 2020[1]. Triple-negative breast cancer (TNBC) is a type of BC that is negative for the progesterone receptor, estrogen receptor, and human epidermal growth factor receptor 2, accounting for 15–20% of all BC. As the predominant type of BC, TNBC is characterized by high aggressiveness, poor prognosis, and high recurrence rate, usually in younger women and with an increasing rate of mortality[2–4]. Approximately 45% of TNBC patients develop brain or other metastases[3]. What's worse, TNBC is insensitive to both hormonal and targeted therapies[5], and chemotherapy is virtually the only available option, but it soon develops resistance. It is a challenging and important clinical issue to find accurate and effective targets for TNBC treatment. In recent years, immunotherapy has thrown light on TNBC patients. Immune checkpoint inhibitors (ICIs) programmed death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1) have improved outcomes for some TNBC patients[6]. ICIs could significantly improve event-free survival even in patients who did not reach pathological complete response. However, no predictive biomarker is currently available for the selection of patients most likely to benefit from ICI. Due to economic costs and potential risk of permanent immunotoxicity, only part of patients can be cured by chemotherapy alone; whereas some patients with high tumor burden still have dismal prognosis. Therefore, research should focus on selecting the appropriate population for TNBC immunotherapy and maximizing the potential benefits of ICIs.

The Global Burden of Disease Study 2019 shows that depression is the top ten leading drivers for increased global burden of disease[7]. In the U.S., major depressive disorder (MDD) has a lifetime prevalence of 21% among women[8] and is the leading cause of suicide. MDD may significantly shorten life expectancy partly due to suicide and an increased susceptibility to major illnesses including cardiovascular disease, stroke, autoimmune diseases, and cancer[9–11]. MDD may not only worsen the course of the above medical disorders but also deteriorate treatment outcomes[12]. There are currently no

useful predictors of depression clinically, and such biomarkers are of concern because persistent depression may bring about increased resistance to treatment and increased risks of substance abuse and suicide. Notably, the prevalence of depression is very high in patients with autoimmune diseases, and depressed patients with increased inflammatory markers may be relatively resistant to treatments[13]. Evidence suggests that immune-related genes participate in the pathophysiology of MDD and that enhanced cerebral pro-inflammatory levels induce depression. Recent genome-wide studies have shown that processes (e.g., IL-6 signaling or natural killer cell pathways) associated with immune responses are greatly enriched in MDD patients [14]. Several studies have confirmed that MDD affects the function of the immune system (cellular and humoral immunity), increases the risk of BC recurrence and metastasis, shortens survival time, and enhances mortality rates[15, 16]. In addition, fatigue, pain, loss of appetite, or sleep disturbances in BC patients may be incorrectly attributed to physical illnesses rather than mental disorders.

Past studies have widely recognized that MDD affects the quality of life and prognosis of TNBC patients, thereby increasing the risk of death[17]. Stress-induced changes in hematopoiesis lead to mononucleosis, neutrophilia, and lymphopenia, and consequently upregulate pro-inflammatory levels in immune-related peripheral tissues. This peripheral inflammation can trigger psychiatric symptoms, metabolic syndrome, immunosuppression, and other psychiatric complications[18]. Many articles have addressed the potential mechanisms by which stress signals are transmitted from the central nervous system (CNS) to immune cells to modulate stress-related behaviors and psychiatric complications[19–22]. It would be of great interest to uncover potential biomarkers for both diseases. Thus, determining different clustering features and MDD-related features can effectively predict TNBC patients' prognosis and response to immunotherapeutic interventions. MDD signature was identified by differential analysis based on the MDD scRNA dataset GSE144136. TNBC samples were obtained from TCGA, GEO, and METABRIC databases. The expression and mutations of MDD signature genes in TNBC were analyzed and an MDD-related prognostic signature was developed based on the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) database, which was validated by the GSE58812 and TCGA databases. We finally analyzed the high and low-risk groups in functional enrichment, cancer stemness, immune cell infiltration, response to immunotherapeutic interventions, mutation frequency, and chemotherapy resistance. The results illustrated that the MDD-related signature was linked to immune cell infiltration and could predict treatment responses and outcomes in TNBC.

# 2. MATERIALS AND METHODS

# 2.1 Study Design and Data Collection

Single-cell mRNA sequence (scRNA-seq) data from 17 MDD patients and 17 controls were downloaded from GSE144136 in the GEO database (www.ncbi.nlm.nih.gov/geo) [23]. RNA sequencing data and clinical annotations of TNBC patients were obtained from the METABRIC ((http://www.cbioportal.org/) and used as the training cohort. The GSE58812 microarray dataset was downloaded from GEO [24] and

the RNA-Seq data of TNBC samples were procured from TCGA (https://portal.gdc.cancer.gov/repository). Detailed information is exhibited in Table 1.

Table 1
Information of datasets

| Dataset | Platform | Origin | Sample | | Species |
|---------|----------|--------|--------|---------|---------|
| | | | Experimental | Control | |
| GSE144136 | GPL20301 | Post-mortem dorsolateral prefrontal cortex | 17 | 17 | Homo sapiens |
| GSE58812 | GPL570 | neoplasms | 107 | / | Homo sapiens |
| TCGA TNBC | Illumina | Invasive Ductal Carcinoma of Breast | 112 | / | Homo sapiens |
| METABRIC | Illumina | Invasive Ductal Carcinoma of Breast | 320 | / | Homo sapiens |

## 2.2 Selection and Analysis of the Differential Expression of MDD-Related Genes

MDD scRNA-seq data from GSE144136 were analyzed by Seurat (https://github.com/satijalab/seurat) [23]. Cells with less than 200 genes or more than 2,500 genes and more than 5% of mitochondrial gene fragments were screened. Seurat's functions of NormalizeData and ScaleData were utilized for normalization and scaling of count data after the remaining cells were merged into one gene expression matrix. RunUMAP and Findclusters functions were utilized for dimension reduction and cell cluster identification. After that, cell clusters were annotated by the SingleR R package. "FindAllMarkers" and "FindMarkers" functions were applied for Wilcoxon tests in astrocytes between MDD patients and controls to find differentially expressed genes (DEGs). Afterward, a protein-protein interaction (PPI) network was established for DEGs using the STRING database [25]. The R package "clusterProfiler" [26] was adopted for functional enrichment analyses with KEGG and Gene Ontology (GO), with a cutoff value of p < 0.05.

## 2.3 Identification of a Prognostic MDD-related Gene Signature in TNBC

The prognostic performance of dysregulated MDD genes was estimated via univariate Cox regression analysis (p < 0.05) in the METABRIC dataset. Subsequently, the stepwise Akaike information criterion (stepAIC) method from the MASS package (version 26) was utilized to refine the prognostic gene set and construct a prognostic model. The risk score was graded based on the normalized expression levels of genes (Expi) and regression coefficients (Coei):

$$\text{Riskscore} = \sum_{i=1}^{N} (Expi \times Coei)$$

TNBC patients were assigned to high and low-risk groups based on the median cutoff. The 'survminer', 'survival', and 'survivalROC' R packages were then employed for Kaplan−Meier and ROC curve analyses to assess the prognostic performance of novel gene signatures. Cox regression analyses were conducted to evaluate the prognostic independence of MDD-related risk scores with other clinical indexes in TNBC patients. A survival prediction nomogram was established by incorporating significant risk factors and its accuracy was assessed with the calibration curves and decision curve analysis (DCA).

## 2.4 Functional Enrichment Analysis

DEGs between the high and low-risk patients were determined by |logFC| > 0.5 and P < 0.05 and then selected for GO analysis with the 'clusterProfiler' R package [27]. Gene Set Enrichment Analysis (GSEA) of the KEGG pathway was performed using the "clusterProfiler" R package [28], with the threshold of |NES| > 1, NOM p-value < 0.05, and q-value < 0.25.

## 2.5 Relationship of MDD Prognostic Signature with TME in TNBC

The 'ESTIMATE' R package was employed for calculating the stromal score, immune score, and ESTIMATE score to estimate the TME (tumor microenvironment) composition. For further analysis, immune checkpoints and HLA-related gene expression matrix were extracted for differential analysis. To identify the mutational profiles of TNBC patients, the mutation annotation format was created with the "maftools" package [29].

## 2.6 Immune infiltration analysis

The proportion of immune cells was determined for patients in the low-risk and high-risk groups using CiberSort, a computational method that identifies different immune cell proportions by tissue gene expression profiles. The TIMER database (https://cistrome.shinyapps.io/timer/)) includes 10 of 32 cancer types from the Cancer Genome Atlas. 897 samples to estimate the abundance of immune infiltrates (26). Immune cell infiltration analysis was performed using the "CiberSort" R script and the Timer 2.0 database. Using the heatmap R package, a heatmap was created showing the infiltration of 28 immune cells into the body. In the box plots, we visualized the differences between the high and low risk groups regarding the proportion of different types of immune cells.

## 2.7 Stemness Signatures Analysis

According to the most comprehensive and up-to-date set of published stemness signatures defined by RNAi screening, gene expression profiling, target gene sets of transcription factors, literature, and computational summaries (Additional file 2: Supplementary Table 1), 26 stemness gene sets were recruited from StemChecker (http://stemchecker.sysbiolab.eu/). Next, ssGSEA was applied for quantifying stemness enrichment scores via the GSVA R package and for differential analysis.

## 2.8 Drug Sensitivity Prediction

The 'pRRophetic' R package [30] was employed for drug sensitivity estimation. The ridge regression was implemented to calculate the IC50 value based on the GDSC database.

## 2.9 Statistical Analysis

All statistical analyses were done using R software (v4.3.1). Wilcoxon test was utilized for pairwise comparisons (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). Kaplan−Meier method and log-rank test were applied for survival analyses. The optimal cutoff value of the stemness and risk scores was examined with the "surv_cutpoint" function of the survminer R package (v0.4.6). P value < 0.05 presented statistical significance.

## 3. RESULTS

## 3.1 scRNA-seq analysis reveals marker genes for MDD

The flow chart of the study design is exhibited in Figure.1. After batch effect removal, the first 2000 highly variable genes in the cells were obtained (Figure.2A). Principal component (PC) values were determined with the elbow plot function (Figure.2B), which indicated that the optimal PC value was 10 because it was the last point where the percentage change in variation exceeded 0.1%. A resolution of 0.5 was confirmed by clustree. UMAP downscaling visualized individual cells into 22 clusters (Figure.2C), which were then annotated into 8 cell types by SingleR annotation (Figure.2D). Among them, 298 genes were characterized genes because their levels differed significantly from those in normal cell types (Figure.2E). This suggests that astrocytes are predominant cells in MDD tissues. Astrocytes are the major type of glial cells in the mammalian CNS and are strongly associated with depression.

## 3.2 MDD key genes mutated and expressed in TNBC

298 DEGs were obtained and then subjected to PPI analysis, which showed that the core genes were densely connected and closely related (Figure.3A). Summary analysis of MDD core gene expression mutations in the TCGA-TNBC cohort revealed a high incidence of mutations in the first 20 genes of MDD (Figure.3B). In the TCGA cohort, 39 out of 98 samples (39.8%) had significant gene alterations, and the gene with the highest mutation frequency was RYR2. One study showed that LINC01194 activated the Wnt/β-catenin pathway and accelerated TNBC progression by recruiting NUMA1 to stabilize UBE2C mRNA and enhance RYR2 ubiquitination. Additionally, 20 key MDD genes exhibited significant CNV alterations in TNBC patients (Figure.3C). The function and significant KEGG pathways of 298 MDD core genes were further investigated (Figure.3D), and GO and KEGG pathway studies in MDD gene modules were shown to correlate with neurological class functions such as brain and behavior.

## 3.3 Prognostic characteristics and prognostic value of MDD signature genes in TNBC patients

Univariate Cox regression analysis was carried out to identify MDD genes with prognostic significance to establish a predictive model. The initial analysis unveiled 28 genes with significant prognostic capacity

(p < 0.05). To streamline the model with fewer genes, we executed stepAIC analysis and ultimately selected 10 MDD genes to construct the prognostic model. The formulation was as follows: Risk score = (-0.2062) * PTGDS + (1.8077) * FGF14 + (0.4844) * CRLF3 + (-0.2984) * ST6GALNAC5 + (0.1002) * CKB + (1.0944) * CDH12 + (0.4444) * PKM + (0.3172) * RHOB+ (0.3462) * GNB2 + (0.2333) * HIP1R. Subsequently, TNBC patients were allocated into two groups as per their computed risk scores. Kaplan-Meier method revealed that TNBC patients with high-risk scores had poorer OS probabilities than their low-risk counterparts from the METABRIC-TNBC dataset (median time = 75.3 months vs. 292.7 months, P < 0.0001, Figure.4A). Risk score distribution and survival outcomes are presented in Figure.4A. To verify the robustness, we extended our analysis to two independent validation groups: the TCGA-TNBC cohort and the GSE58812 cohort. In both validation cohorts, patients with high-risk scores had poorer OS than those with low-risk scores (TCGA-TNBC: median time = 98.8 months vs. 115.7 months, P = 0.012, Figure.4B; GSE58812: median time = 54.5 months vs. 77.2 months, P = 0.00052, Figure.4C). These data affirm the robust performance of the 10-gene prognostic model in predicting the TNBC prognosis across multiple datasets.

## 3.4 Clinical characteristics of TNBC and construction of related prognostic indicators

Univariate and multivariate Cox analyses identified age, lymph node, and risk score as independent prognostic indicators for TNBC patients (Figure.5A, B). To make the model clinically applicable and feasible, we established a Nomogram based on the METABRIC cohort with age, lymph node, and risk score as predictors of overall survival (Figure.5C). The Nomogram-based low-risk group manifested a better prognosis (Figure.5D). The AUC of the combined model for 1-, 3- and 5-year survival were 0.729, 0.684, and 0.753, respectively (Figure.5F), which were all roughly at 0.7. Furthermore, the calibration curve manifested that the nomogram could make accurate predictions (Figure.5G). Additionally, DCA (Figure.5E) elicited that the nomogram better predicted the 3- and 5-year OS, providing more net clinical benefits than the 1-year OS. Overall, our developed nomogram demonstrates predictive power and clinical applicability in assessing the prognosis of TNBC patients based on these important clinical parameters.

## 3.5 MDD prognostic signature genes are expressed in immune cells and promote TNBC development

Besides, the low-risk group had higher stromal score, immune score, and estimate score than the high-risk group (Figure.6A). In the TME, the ratio of immune and stromal cells in the tumors could significantly affect the prognosis. The results verified that the low-risk group had a better prognosis than the high-risk group. ICIs have been increasingly utilized in clinical settings for antitumor immunotherapy. Variations in ICI expression among high-risk and low-risk populations could lead to different responses to ICIs. Our investigation revealed that certain ICIs were significantly expressed between the high- and low-risk TNBC patients, with low-risk patients having higher levels and being more suitable for immunotherapy (Figure.6B). HLA phenotypes have great influences on the efficacy of immunotherapy drugs. The greater the HLA diversity, the more types of neoantigens can be delivered. This study found 12 significant

differences in phenotypes (Figure.6C), suggesting that immunotherapy are more effective in low-risk patients. The stemness index was elevated in high-risk patients, suggesting higher intratumor heterogeneity (Figure.6D). The IC50 of the four drugs was assessed using the GDSC dataset, which demonstrated that high-risk patients had higher IC50 values for the four complexes, suggesting less sensitivity to chemotherapeutic agents, Camptothecin, and ARTA (Figure.6E). This implies that the high-risk patients are insensitive to conventional therapeutic modalities, and there is a need for the development of a completely new therapeutic approach. Of note, increased levels of stemness-related factors are correlated with tumor recurrence, drug resistance, and cell proliferation. Tumor-loaded mutation scores were also markedly higher in the high-risk patients (Figure.6F). In addition, we found that in (Figure.6G), the low-risk group had better immune cell infiltration compared to the high-risk group, suggesting that the low-risk group is more likely to benefit from immunotherapy.

## 3.6 MDD Characterization Genes Expression and Mutations in TNBC

Figure.7A manifests the localization of the 10 characterized genes on human chromosomes. Pearson analysis indicated that PTGDS expression showed a strong positive correlation with CRLF3 expression and a strong negative correlation with ST6GALNAC5 expression, and PKM expression showed a strong negative correlation with CRLF3 expression (Figure.7B). GO semantic similarity analysis showed that FGF14 had the highest functional similarities (Figure.7C), and the higher semantic similarity indicated that the gene played a more important role in the function. As shown in the PCA plot, gene expression patterns between the high-risk and low-risk groups were quite different (Figure.7D). The expression differences of 10 characterized genes were comprehensively evaluated to explore the molecular characteristics. The results exhibited differences in the characterized gene expression profiles and clinical features between high-risk and low-risk groups (Figure.7E). To further elucidate the molecular mechanism of 10 characterized genes, the Network analyst online tool was used to predict the interaction network of miRNA-characterized genes-transcription factors (Figure.7F).

## 3.7 Functional enrichment analysis of Low-Risk and High-Risk populations

GO BP results suggested that high-risk populations had poorer outcomes but more active immune functions, indicating higher sensitivity to immunotherapies (Figure.7G). To figure out the potential differences in biological functions, we performed GSEA and identified the 10 most critical enriched pathways (Figure.7H). The low-risk populations were mainly related to immune function, including allograft rejection, antigen processing and presentation, intestinal immune network for IgA production, autoimmune thyroid disease, natural killer cell-mediated cellular toxicity, primary immunodeficiency, cytokine-cytokine receptor interactions, inflammatory bowel disease, Th1 and Th2 cell differentiation, and Th17 cell differentiation.

## 4. DISCUSSION

TNBC is the most malignant type of BC and there is a high rate of distant metastases and lack of accurate and effective therapeutic targets. Is TNBC progression associated with certain genes that characterize brain disease? To date, many studies have suggested an extremely high incidence of BC and anxiety-depressive disorders, with 84% of patients with advanced BC experiencing anxiety-depression[31]. For MDD, mutations have been observed in the genetic region of the FKBP5 allele, which involves abnormal functions of the hypothalamic-pituitary-adrenal (HPA) axis and links to enhanced blood cortisol and plasma adrenocorticotropic hormone[32]. Enhanced cortisol levels and malfunctioning of inhibitory mechanisms change the communication between the HPA axis and/or the CNS and the immune system[33], thus, inducing an inflammatory process[34]. Immunization processes are associated with the pathophysiology of both MDD and TNBC. Only a few have examined the relevance of MDD and TNBC in terms of pathogenesis, and so we propose that MDD signature genes may be expressed in immune cells and promote TNBC progression, thus affecting patient prognosis and immunotherapy responses. In recent years, integrated bioinformatics analysis based on massive data has been increasingly used to explore new genes and potential diagnostic or prognostic biomarkers to provide more information for disease pathogenesis and prospective treatment [35, 36]. In this study, we employed various bioinformatics analysis methods to unveil for the first time the role of MDD signature genes in TNBC and elucidate the relationship between MDD and TNBC from an immune perspective. This novel perspective helps us understand how MDD signature genes affect the prognosis and development of TNBC and provides predictive biomarkers for the stratification of patients most susceptible to ICIs.

The highly heterogeneous characterization of TNBC leads to different clinical outcomes and treatment sensitivities[37]. Hence, we developed a new MDD-related marker that has enabled risk stratification and personalized treatment. Brain nuclear tissues were annotated from 34 MDD suicide patients from GSE144136. Twenty-two cell clusters were annotated into eight cell types, namely Astrocyte, Endothelial cells, Gametocytes, Macrophages, Neuroepithelial cells, Neurons, and Platelets. Our analysis suggested that astrocytes are the major cell type in MDD and can be used as the characteristic cells of MDD. Astrocytes can respond to and promote inflammatory signals and regulate multiple life processes in the CNS, both physiologically and pathologically[38]. Emerging evidence suggests that astrocyte dysfunction is implicated in MDD pathogenesis. Activated astrocytes facilitate the production of pro-inflammatory cytokines, like interleukin IL-1β and TNF-αto induce depressive symptoms [39−43]. Of note, 298 genes were revealed as signature genes because their levels were substantially different from those in normal cell types. 298 MDD signature genes had significant (39.8%) genetic alterations in the TCGA-TNBC cohort. The more mutations there are in tumor cells, the more likely they are to produce aberrant proteins, thus increasing the recognition probability by the immune system and activating the body's immune response[44]. These data suggest that TNBC populations based on MDD signature genes may benefit from immunotherapy.

We calculated the risk scores of the patients and divided the 320 samples of the METABRIC dataset into high- and low-risk groups as a training set. The survival of the low-risk patients was visibly longer than that of the high-risk patients, similar to the result in the TCGA and GSE55812 datasets. Further clinical analyses in both univariate and Cox analyses confirmed age, lymph nodes, and risk scores as

independent prognostic factors for TNBC, and these results further validated our inference. TNBC is considered an early-onset subtype of BC because of its strong immune escape capacity[45, 46].

The inflammatory hypothesis of MDD is also called the monocyte/macrophage theory, and cells of this lineage are primary producers of pro-inflammatory cytokines[47, 48]. Some researchers found overexpressed inflammatory genes in monocytes of MDD patients[49, 50]. In patients with solid tumors, responders exhibit a'hot' ('immune-inflamed') phenotype, characterized by T lym-phocyte infiltration, whereas nonresponders may exhibit a'cold' ('immune-desert'/'immune-ex-cluded') phenotype, characterized by the absence or exclusion of T cells in the tumor parenchyma[51], the TNBC high- and low-risk groups distinguished by the characteristic inflammatory genes based on MDD have significant differences in T-lymphocyte infiltration between the two groups, which can help to screen for immunotherapy benefit. Consistently, our results showed higher stromal score, immune score, and proportion of immune cells in the low-risk group. The microenvironment of BC is an intricate integrated system that can be classified into an immune cells-dominated TME and a fibroblasts-dominated non-immune TME. In this regard, the genetic profiles of immune cells (genes related to gene transcription and proliferation) and tumor-infiltrating lymphocytes (TILs) may be particularly important for tumor progression, clinical response, and prognostic value for TNBC patients with limited therapeutic options and poor prognosis[52]. We detected a landscape of immune infiltration of multiple immune cell types and noticed that the high-risk group had a lower percentage of immune infiltration and was more susceptible to tumor progression and metastasis. Few immune cells are also present in normal breast tissue, but tumor progression is associated with leukocyte infiltration in this area. TNBC is characterized by high proliferation and therefore high levels of TILs, partly due to increased genomic instability and mutational load, thus affecting the immune system to clear cells carrying non-self-antigens[53]. Unsurprisingly, the present study revealed that CD8T and T cells were also mostly enriched in the low-risk populations, and cytotoxic T cells hinted at a favorable prognosis in early TNBC.

Finally, there is an important philosophical cognitive difference that haunts the field. Descartes's interactionism holds that the mind and body are mutually independent entities that can interact with each other. Contemporary neuroscientists are now increasingly aware that mental states can indeed influence peripheral physiological processes, although there is still a significant portion of people who believe that immune and inflammatory markers are merely incidental phenomena and have no causal relationship to the physiology and pathology of mental diseases[13].

## Conclusion

In conclusion, we used scRNA-seq analysis to discover for the first time that MDD signature genes can be expressed in immune cells and promote TNBC progression, which may explain the underlying mechanisms of the reciprocal increase in the incidence of TNBC and MDD.

## Abbreviations

TNBC

triple-negative breast cancer

MDD

major depressive disorder

ScRNA-seq

single-cell mRNA sequence

GEO

Gene Expression Omnibus

TCGA

The Cancer Genome Atlas

DO

disease ontology

KEGG

Kyoto encyclopedia of genes and genomes

GDSC

Genomics of drug sensitivity in cancer

TME

tumor microenvironment

DCA

decision curve analysis

ROC

receiver operating characteristic curve

HLA

human leukocyte antigen

# Declarations

## Funding

## Competing interests

The authors declare no competing interests.

## Availability of data and materials

The datasets analyzed during the current study are available in the GEO database (www.ncbi.nlm.nih.gov/geo), Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) ((http://www.cbioportal.org/), and The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/repository). The accession numbers of GEO datasets are GSE58812 and GSE144136.

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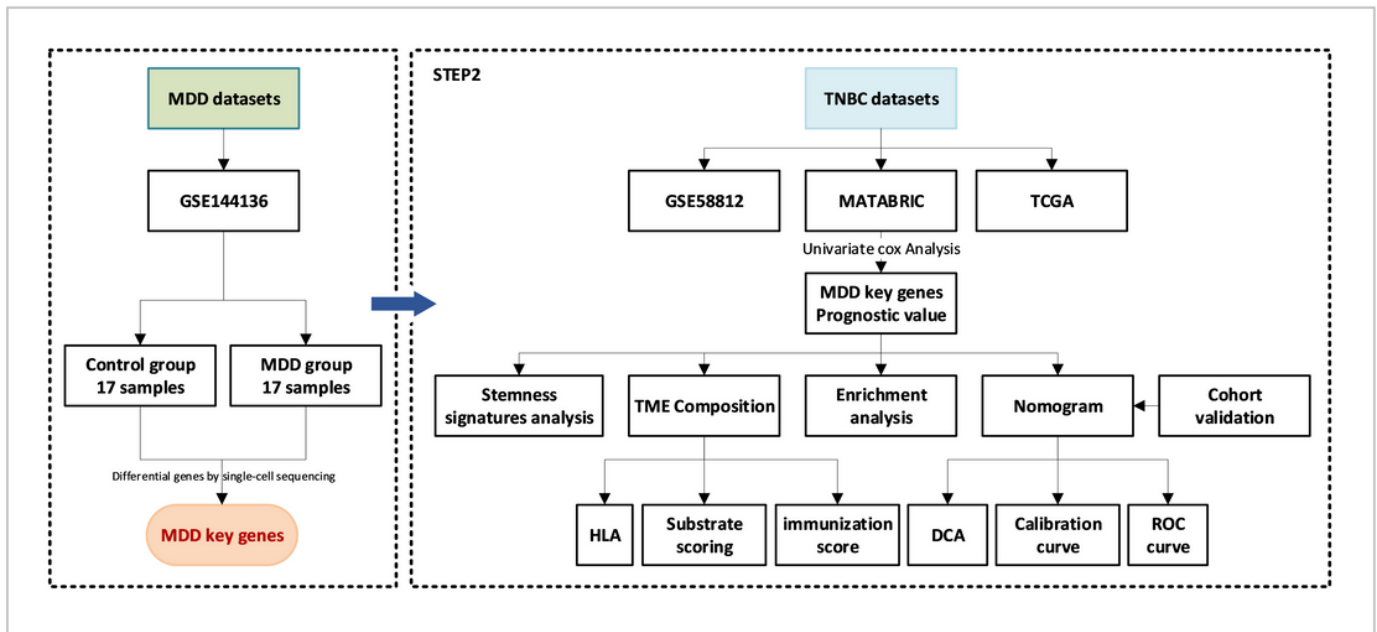
# Figures



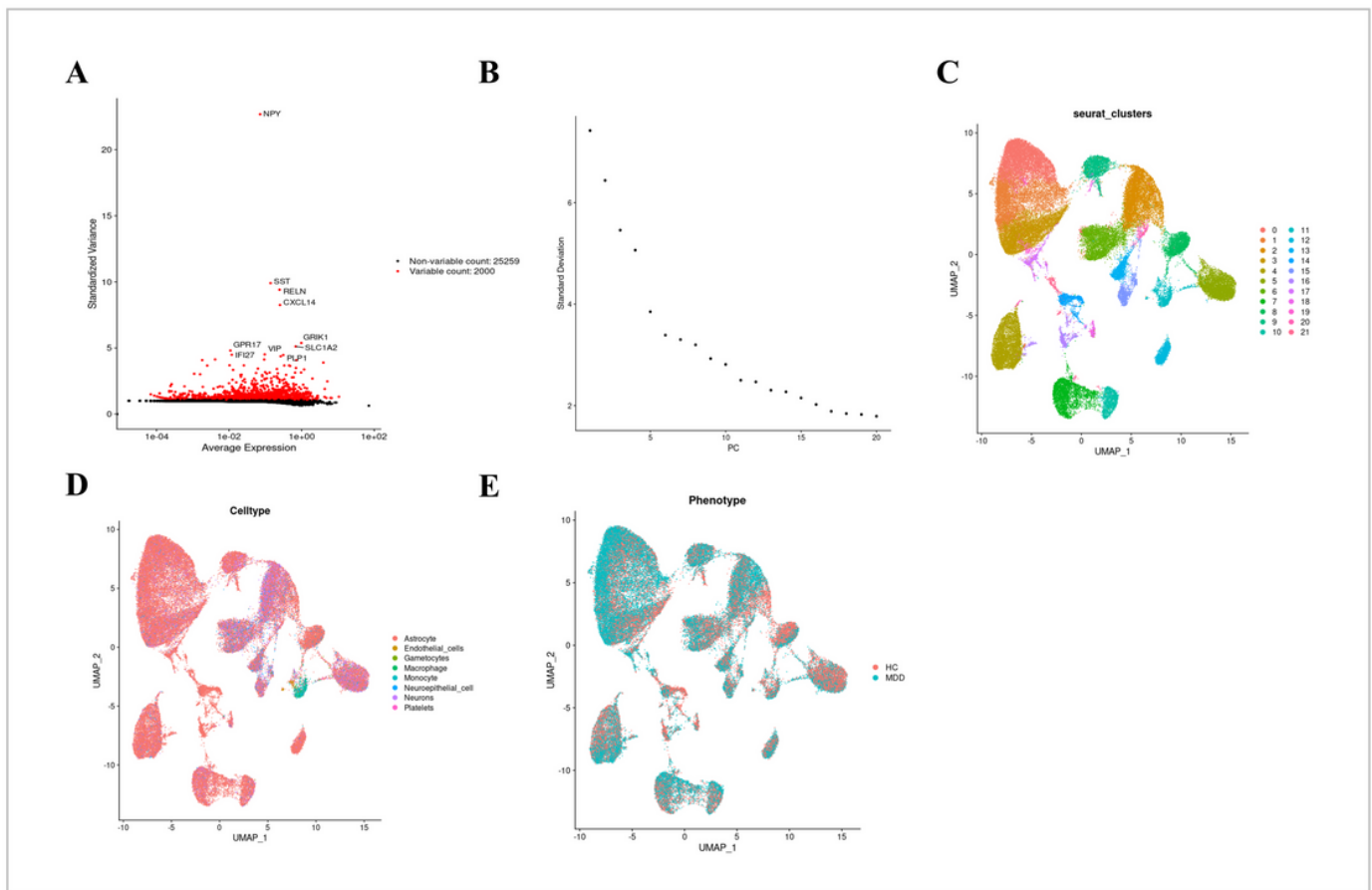## Figure 1

Flow chart of this study design

**Figure 2**

Single-cell cluster analysis of MDD disease and key gene expression in each cluster. (A) Variable characterization plot, 2000 MDD genes with highly variable expression values were selected to represent the cell spectrum. (B) Principal component analysis was performed on 2000 genes (C) The first 10 principal components were selected for cluster analysis, and 22 clusters were obtained by co-clustering; (D) Cellular subgroups were annotated using SingleR, and different colors represented different cell types. (E) Differential expression was done in normal diseases and a total of 298 differentially expressed genes were obtained, detailed differentially expressed genes are shown in Appendix A. *p<0.001; **p<0.001; ***p<0.001.
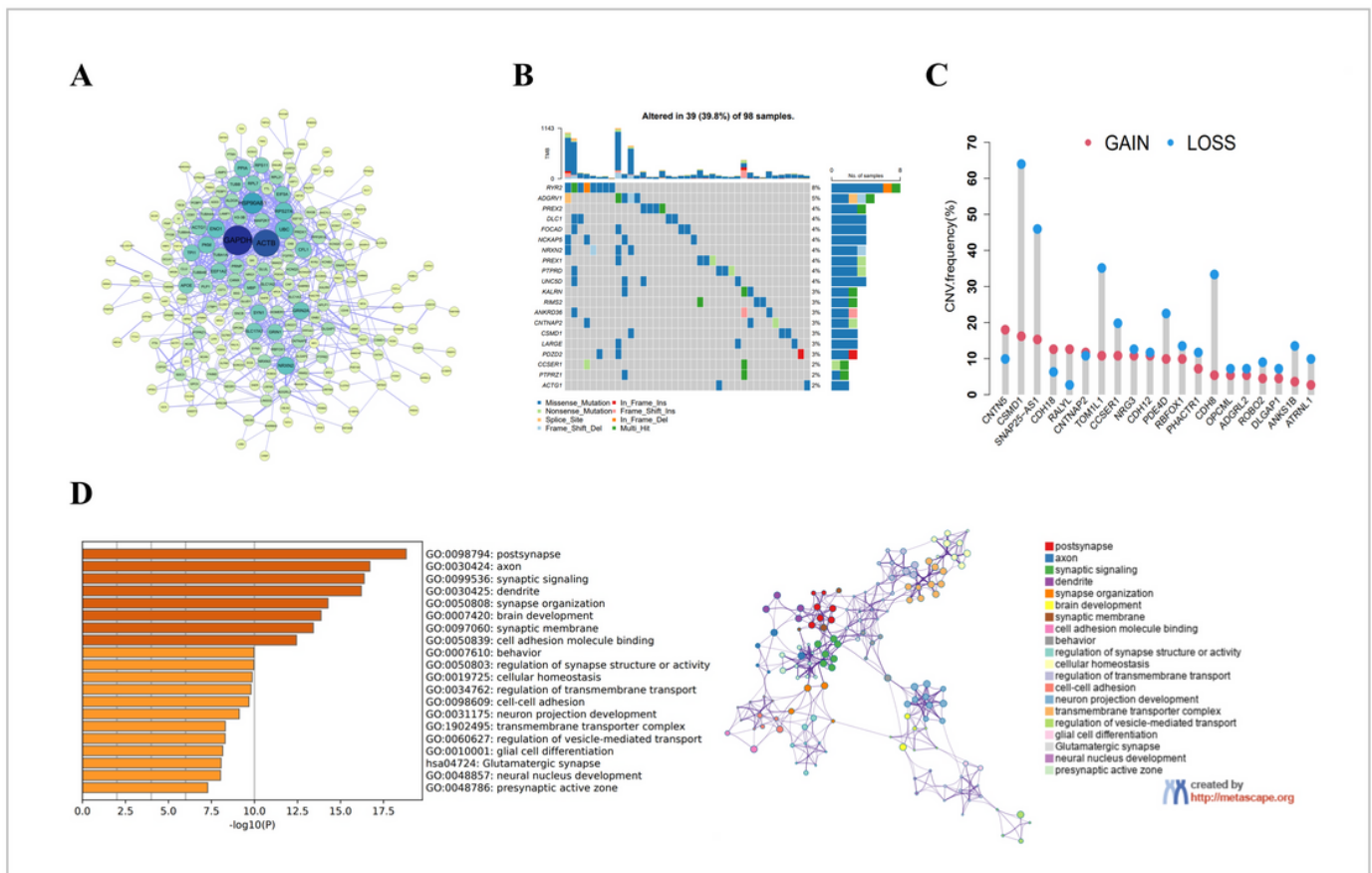
**Figure 3**

MDD core gene function and expression in TNBC (A) PPI analysis of key MDD genes. (B) Frequency of the first 20 MDD core gene expression mutations in 98 TNBC patients in the TCGA database. (C) Frequencies of CNV gain, loss, and non-CNV among TNBC patients on MDD core genes. (D) Gene ontology analysis and KEGG pathway analysis of MDD gene interaction networks from the brown module.
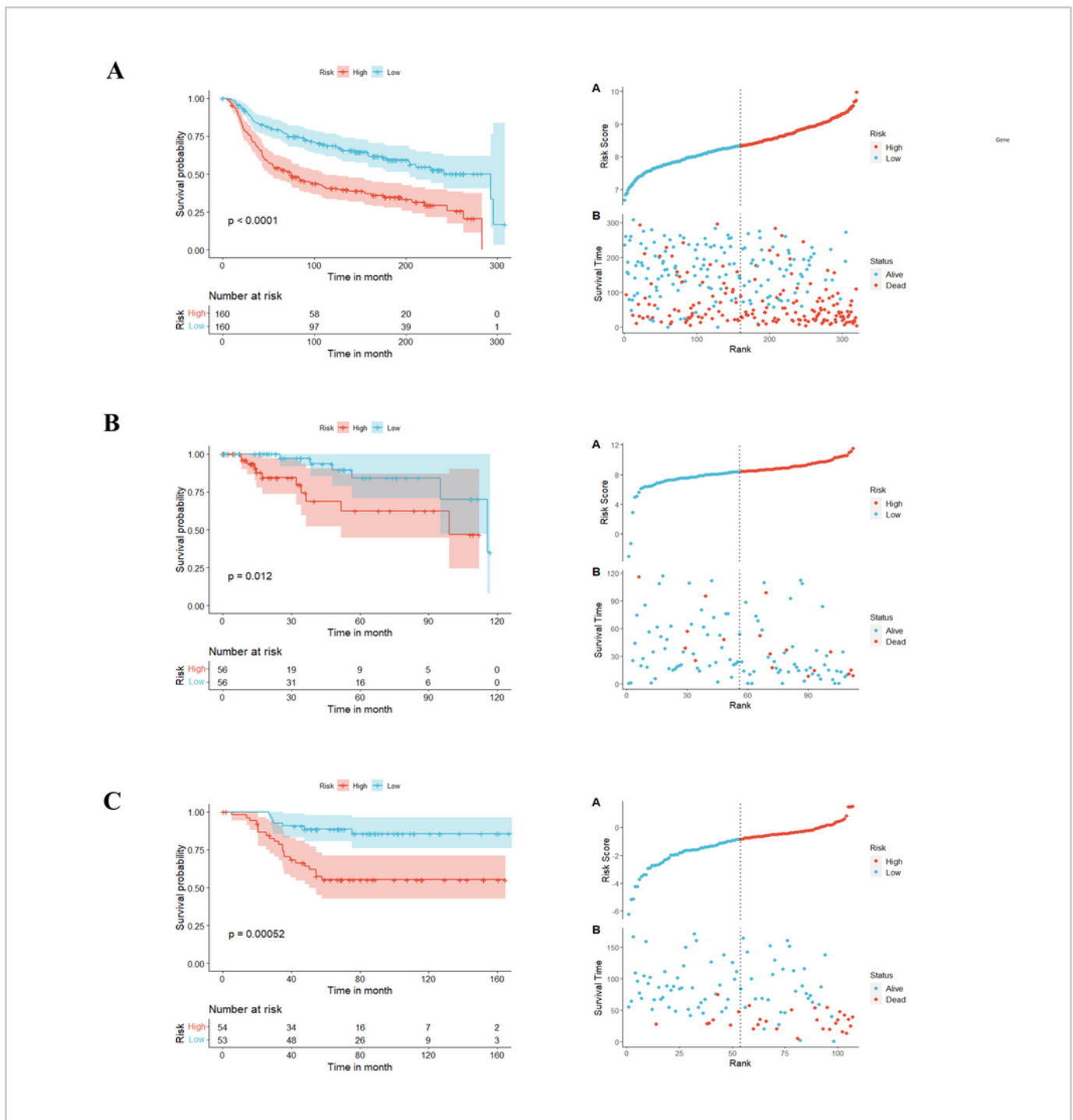
**Figure 4**

Prognostic characteristics of MDD signature genes in TNBC patients and their prognostic value (A) Kaplan-Meier survival curves and Risk Score plots of overall survival (OS) of patients in the high-risk and low-risk groups in the METABRIC-TNBC cohort. (B) Kaplan-Meier survival curves and Risk Score plots of overall survival (OS) of patients in the high-risk and low-risk groups in the TCGA-TNBC cohort. (C) Kaplan-

Meier survival curves and Risk Score plots of overall survival (OS) of patients in the high-risk and low-risk groups in the GSE55812 cohort. *p<0.001; **p<0.001; ***p<0.001.
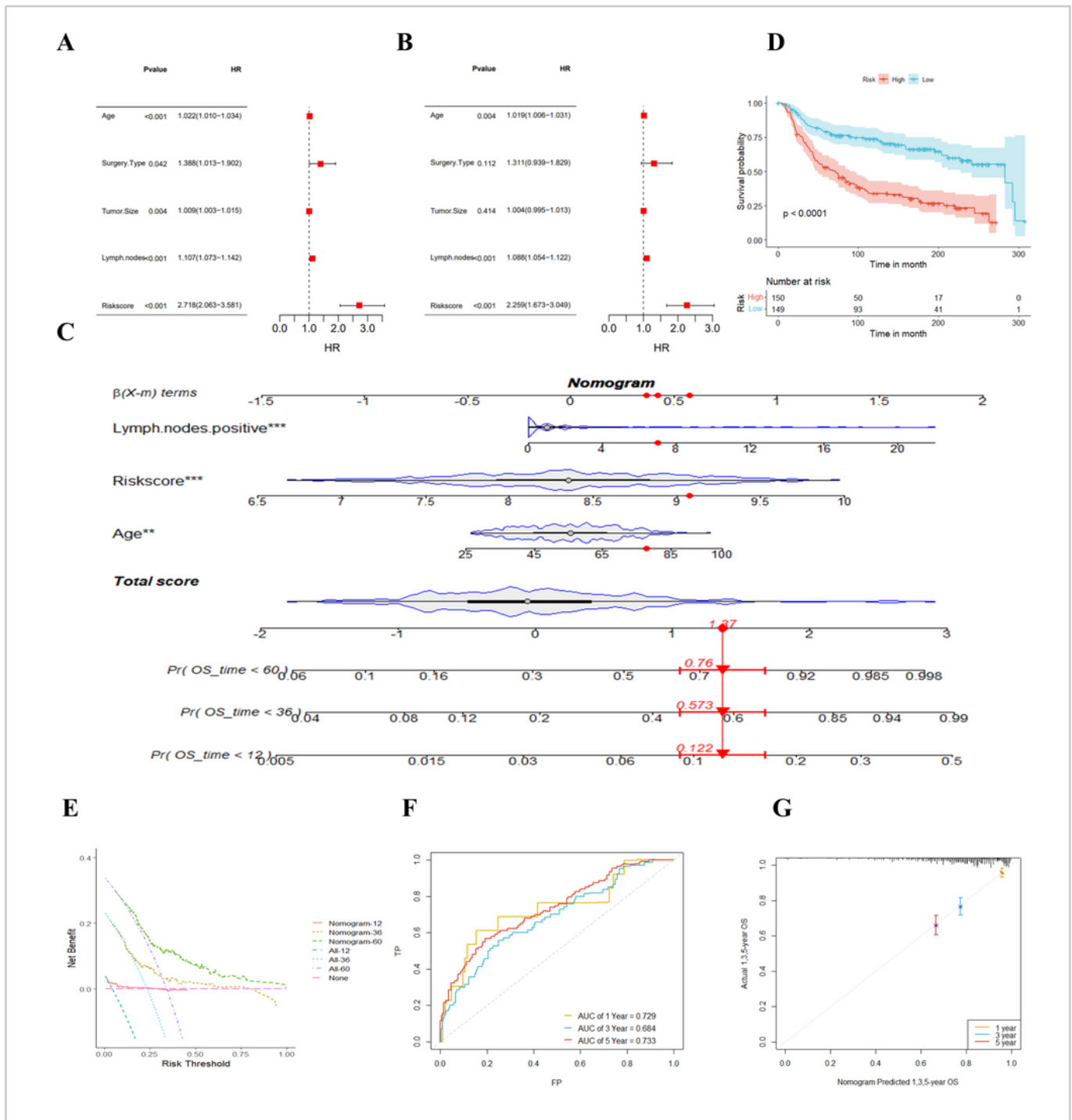


**Figure 5**

Prognostic impact of risk score and clinical characteristics of TNBC patients. (A) Univariate and (B) multivariate Cox analyses assessing prognosis and clinical characteristics including age, type of surgery,

tumor size, lymph nodes, and Riskcore. (C) Column line plot of risk scores and clinical characteristics predicting 1-, 3-, and 5-year survival in the TCGA-TNBC cohort. (D) Kaplan-Meier survival curves based on column-line plots defining overall survival in high and low-risk patients with a validation set of the METABRIC cohort population. (E) Decision curve analysis, a specific method developed to assess the prognostic value of a column line plot strategy, where the column line plot with the greatest net benefit would be the most preferred model. (F) Survival ROC plots to determine the sensitivity and specificity of TNBC survival-related genes as indicators for determining survival. (G) Cralibration curves of the TNBC risk factors nomogram. **p<0.001; ***p<0.001, ****p<0.0001.

**Figure 6**

Immune microenvironment analysis of MDD signature genes in TNBC disease (A) Differences in TME scores between high and low risk groups (B) Differences in immune cell infiltration between high and low risk groups. (C) Differences in human leukocyte antigen cells between high and low risk groups. (D) Differences between high and low risk groups in tumor stem cell expression. (E) Sensitivity of high and low risk groups to different anticancer drugs (F) Differences in tumor mutation load between high and

low risk groups. (G) Immune cell infiltrated in high and low risk scoring groups *p<0.001; **p<0.001; ***p<0.001.
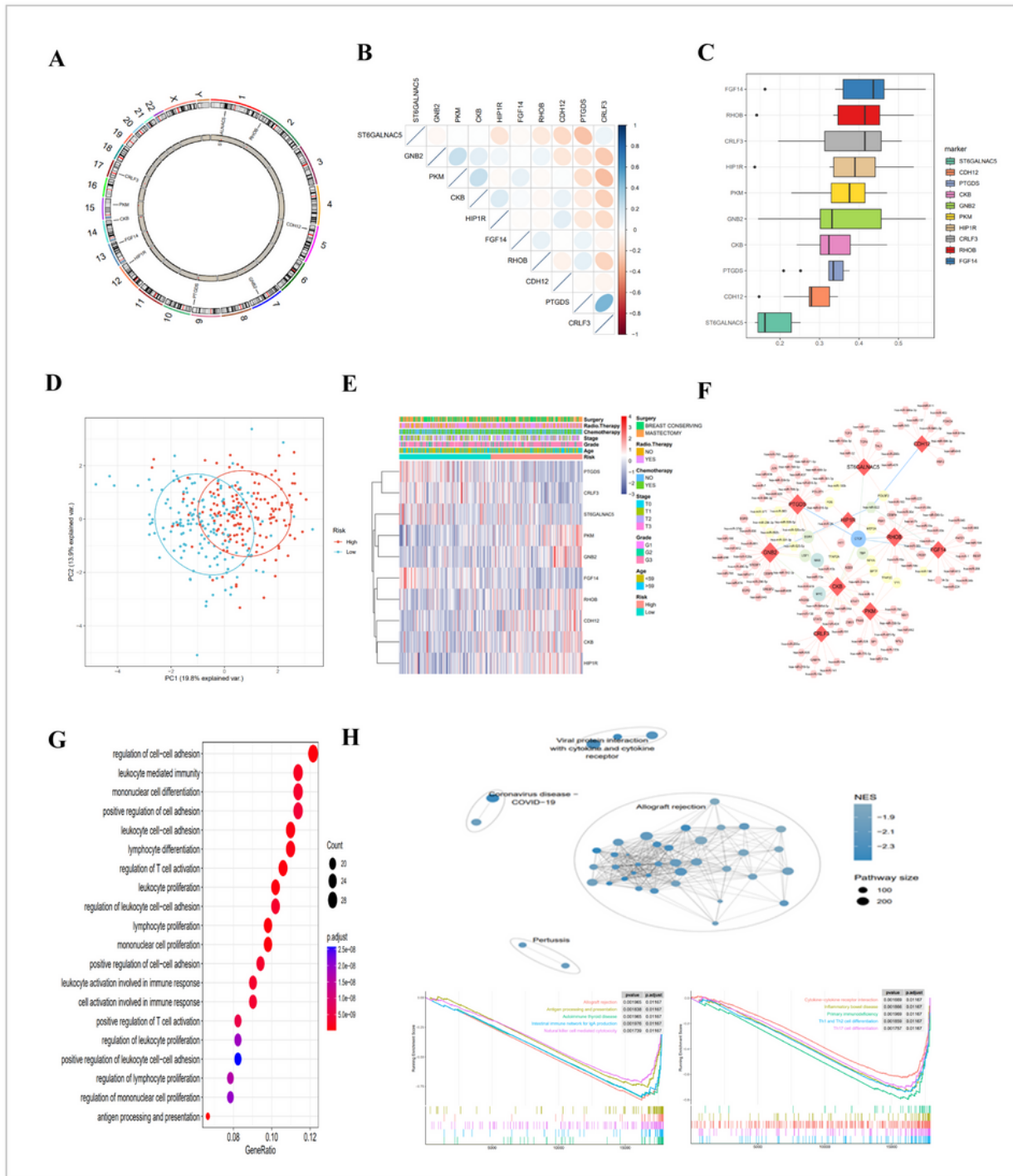


**Figure 7**

Transcriptional alterations and expression of MDD marker genes in TNBC disease and enrichment pathway analysis of risk genes. (A) Localization of CNV alterations on MDD marker genes on

chromosome 23. (B) Correlation study of the expression of 10 MDD marker genes (C) Correlation visualization plot of 10 MDD marker genes in TNBC disease. (D) Principal component analysis showing significant differences between MDD marker high-risk and low-risk cohorts (E) Heatmap showing differences in clinical information and expression between MDD marker high-risk and low-risk cohorts (F) Regulatory network map of miRNA-transcription factors for 10 key genes. (G) Bubble diagram showing the results of KEGG enrichment analysis. (H) GSEA analyses of different KEGG pathways were clustered in the high-risk and low-risk groups. *p<0.001; **p<0.001; ***p<0.001.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.csv