Research Paper

Low expression of endoplasmic reticulum stress-related gene SERP1 is associated with poor prognosis and immune infiltration in skin cutaneous melanoma

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ABSTRACT

Stress-associated endoplasmic reticulum protein 1 (SERP1) is a gene induced by endoplasmic reticulum (ER) stress and a major contributor to multiple tumor types. Skin cutaneous melanoma (SKCM) is a highly aggressive and fatal cancer with poor treatment outcomes after progression. In this study, we evaluated SERP1's role in tumorigenesis, prognosis, and immune infiltration in SKCM. Patients with SKCM had low SERP1 expression. We identified differentially expressed genes between high- and low-SERP1 expression groups and conducted functional, pathway, and gene enrichment analyses. Protein–protein (PPI) and gene–gene interaction (GGI) networks were constructed via STRING and GeneMANIA, respectively. SERP1 mutation information was obtained through cBioPortal; location in the skin was identified through the Human Protein Atlas. Kaplan–Meier analysis revealed an association between low SERP1 expression and overall survival (OS), disease-specific survival (DSS), progress-free interval (PFI) rates, and worse prognosis in patients with multiple clinicopathological features. Cox regression analysis and nomograms further presented SERP1 level as an independent prognostic factor for patients with SKCM. Furthermore, there were significant correlations between SERP1 expression and immune infiltrates; thus, low SERP1 expression is associated with immune cell infiltration and can be considered a poor prognostic biomarker in patients with SKCM.

INTRODUCTION

Skin cutaneous melanoma (SKCM) is a common cancer in young adults and the elderly, accounting for 2% of all cancer diagnoses worldwide each year [1]. Melanocytes and pigment-containing cells can transform malignantly into the disease. The incidence of SKCM has steadily globally increased over the past 50 years, with ~96,000 new cases in 2019 [2]. Although SKCM only accounts for about 10% of all skin cancers, it can account for ~80% of all skin cancer deaths [3] and is arguably the most aggressive and lethal type of skin cancer. In 2019, more than 7,000 people died from SKCM in the United States alone [4]. As one of the most difficult solid tumors to treat, managing SKCM is not only a challenge for doctors but also places a heavy financial burden on society [5]. Given that improved survival rates for patients with SKCM can be achieved through early diagnosis [6], it is necessary to find effective biomarkers for diagnosis and prognosis of the disease.

The development and widespread use of next-generation high-throughput sequencing technologies has made available large-scale omics data, such as The Cancer Genome Atlas (TCGA), that allow an in-depth analysis of candidate tumor biomarkers. An increasing number of studies focus on screening genetic biomarkers of SKCM prognosis that are associated with tumor cell invasion, infiltration, and metastasis. Losing CDKN2A, a gene encoding a tumor suppressor protein, is associated with poor prognosis in melanoma patients [7]. Tumor thickness in patients with metastatic melanoma is associated with elevated serum miR-221, which decreases after tumor excision [8]. Additionally, several studies [9, 10] have reported an association between miRNAs and survival of patients with melanoma; therefore, identifying SKCM genetic markers is important for establishing a comprehensive diagnostic and prognostic model.

Stress-associated endoplasmic reticulum protein 1 (SERP1), a Sec61-associated polypeptide induced by endoplasmic reticulum (ER) stress, stabilizes membrane proteins when they are translocating into the lumen of the ER, which prevents unfolded target proteins from degradation during ER stress [11]. Recently, SERP1 has been reported to play an important role in tumor cell survival. Ma et al. [12] reported that SERP1 is a marker of poor prognosis in patients with pancreatic ductal adenocarcinoma. Additionally, high SERP1 levels are associated with poor outcomes in glioblastoma patients [13]; however, studies on the correlations of SERP1 with SKCM prognosis are still lacking. Therefore, this study used large-scale bioinformatics databases to conduct a comprehensive bioinformatics exploration of SERP1 as a prognostic marker for SKCM and investigated the underlying mechanisms.

RESULTS

SERP1 expression in pan-cancers and SKCM patients

The association between SERP1 expression and clinical characteristics in SKCM patients were list in Table 1. By analyzing The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) database, we obtained the expression of SERP1 RNA in pan-cancer. As Figure 1A revealed, compared to normal tissues, there was significantly different expression of SERP1 mRNA in 33 incorporated cancers except KICH, KIRC and THCA. Because there were only tumor-related samples without normal tissue samples (MESO and UVM) or too few normal tissue samples (SARC having 2 samples) in the database for these three cancer types, these three cancers could not be compared to normal tissue in the pan-cancer comparison and therefore no results are shown. As the target of our study, SERP1 expression was lower in SKCM tumors than in normal tissues ($p = 0.002$, Figure 1B). Then SKCM patients were divided into the high SERP1 expression group and low SERP1 expression group based on the median SERP1 expression. We compared the RNA expression between these two groups. There were 111 RNAs that met the established selected threshold and were recognized as differentially expressed genes (DEGs) (Adjust P-value < 0.05 and absolute log-fold change > 3) (Figure 1C), of which 30 were upregulated (logFC is positive) and 81 were downregulated (logFC is negative). Top 15 up-regulated and down-regulated DEGs were illustrated by heatmaps and included in the table (Figure 1D, 1E and Supplementary Table 2). We also analyzed RNAs correlated with SERP1 expression in SKCM patients and showed the top 50 positively and negatively associated RNAs as heatmaps (Figure 2A, 2B and Supplementary Table 3).

Protein-protein interaction (PPI) and gene–gene interaction (GGI) network analyses of SERP1 in SKCM patients

The PPI network analysis was conducted to explore the potential interactions of SERP1 protein. As Figure 2C presents, the network with interaction nodes and edges was built via STRING, whose top 10 proteins are listed in Supplementary Table 4. The GGI network also showed that the functions of the differentially expressed SERP1 and its associated genes (such as SERP2, XBP1, DNAJB9, SEC23B, ARF4, SLC33A1, BET1, COPB1, SSR1, SRPRA, TMED10, SSR2, PGM3, DNAJB1, HSPA5, C6orf120, TGDS, MGAT2, GOLGA5, TSC22D2) were primarily related to response to topologically incorrect protein, cellular response to unfolded protein, response to unfolded protein, cellular response to topologically incorrect protein, endoplasmic reticulum unfolded protein response, response to endoplasmic reticulum stress and regulation of endoplasmic reticulum unfolded protein response (Figure 2D).

Predicted functions and pathways of the DEGs between high- and low-SERP1 expression in SKCM patients

The functions of the DEGs between high- and low-SERP1 expression groups were predicted by analyzing Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) via R software and Cytoscape software. The different RNA functional of DEGs included three categories: the biological process (BP), molecular function (MF), and cellular component (CC). The top three GO terms of BP, CC and MF functional groups found via R software are present as Figure 3A.

Table 1. The association between SERP1 expression and clinical characteristics in SKCM patients.

Characteristic	Low expression of SERP1	High expression of SERP1	p	Method
n	235	236		
Gender, n (%)			0.138	Chisq.test
Female	81 (17.2%)	98 (20.8%)		
Male	154 (32.7%)	138 (29.3%)		
Race, n (%)			0.771	Fisher.test
Asian	7 (1.5%)	5 (1.1%)		
Black or African American	0 (0%)	1 (0.2%)		
White	224 (48.6%)	224 (48.6%)		
Age, n (%)			0.056	Chisq.test
<=60	115 (24.8%)	137 (29.6%)		
>60	116 (25.1%)	95 (20.5%)		
Weight, n (%)			0.284	Chisq.test
<=70	36 (13.9%)	41 (15.8%)		
>70	100 (38.6%)	82 (31.7%)		
Height, n (%)			0.753	Chisq.test
< 170	64 (25.2%)	54 (21.3%)		
>=170	70 (27.6%)	66 (26%)		
BMI, n (%)			0.590	Chisq.test
<=25	42 (16.7%)	42 (16.7%)		
>25	91 (36.3%)	76 (30.3%)		
T stage, n (%)			0.004	Chisq.test
T1	17 (4.7%)	24 (6.6%)		
T2	34 (9.3%)	45 (12.4%)		
T3	45 (12.4%)	46 (12.6%)		
T4	98 (26.9%)	55 (15.1%)		
N stage, n (%)			0.192	Chisq.test
N0	126 (30.4%)	109 (26.3%)		
N1	31 (7.5%)	43 (10.4%)		
N2	28 (6.8%)	21 (5.1%)		
N3	25 (6%)	31 (7.5%)		
M stage, n (%)			0.076	Chisq.test
M0	219 (49.4%)	199 (44.9%)		
M1	8 (1.8%)	17 (3.8%)		
Pathologic stage, n (%)			< 0.001	Chisq.test
Stage I	32 (7.8%)	45 (10.9%)		
Stage II	90 (21.8%)	50 (12.1%)		
Stage III	79 (19.2%)	92 (22.3%)		
Stage IV	8 (1.9%)	16 (3.9%)		
Radiation therapy, n (%)			0.031	Chisq.test
No	200 (43.1%)	183 (39.4%)		
Yes	31 (6.7%)	50 (10.8%)		
Tumor tissue site, n (%)			0.658	Chisq.test
Extremities	96 (22.9%)	101 (24.1%)		
Trunk	92 (22%)	79 (18.9%)		
Head and Neck	22 (5.3%)	16 (3.8%)		
Other Specify	7 (1.7%)	6 (1.4%)		
Melanoma ulceration, n (%)			0.361	Chisq.test
No	76 (24.2%)	71 (22.6%)		
Yes	96 (30.6%)	71 (22.6%)		
Melanoma Clark level, n (%)			0.010	Fisher.test
I	6 (1.9%)	0 (0%)		
II	9 (2.8%)	9 (2.8%)		
III	30 (9.3%)	47 (14.6%)		
IV	94 (29.2%)	74 (23%)		

V	32 (9.9%)	21 (6.5%)		
Breslow depth, n (%)			0.002	Chisq.test
<=3	85 (23.6%)	100 (27.8%)		
>3	110 (30.6%)	65 (18.1%)		
Age, median (IQR)	61 (51, 72)	56 (45.75, 69.25)	0.003	Wilcoxon

Abbreviations: SERP1, Stress associated endoplasmic reticulum protein 1; SKCM, Skin Cutaneous Melanoma; IQR, interquartile range.

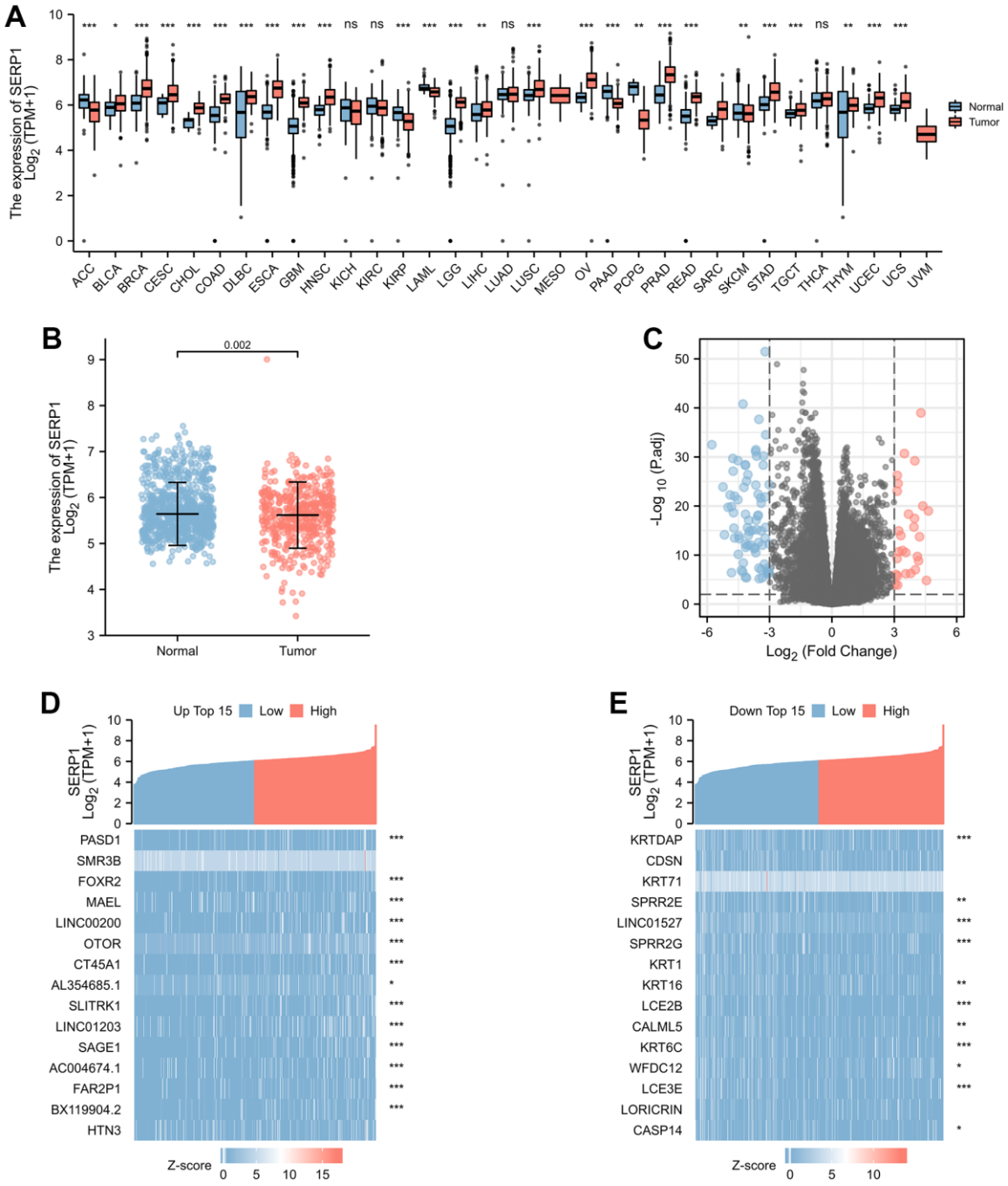

Figure 1. SERP1 expression in cancers. (**A**) SERP1 expression in different cancers and normal tissues in TCGA and GTEx pan-cancer data, ns, $p \geq 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, (**B**) The SERP1 expression in SKCM and normal tissues, (**C**) The volcano plots of DEGs between high and low SERP1 expression groups, (**D**) The heatmap of top 15 up-regulated DEGs, (**E**) The heatmap of top 15 down-regulated DEGs.

The BP ontology included keratinization, epidermis development and keratinocyte differentiation. In the CC ontology, most genes were connected with cornified envelope, intermediate filament and keratin filament. In addition, the structural constituent of epidermis, structural constituent of cytoskeleton with serine-type endopeptidase inhibitor activity made up the majority of MF ontology. Furthermore, the KEGG pathway analysis showed that the DEGs were most closely correlated to Estrogen signaling pathway, GnRH signaling pathway and Inflammatory mediator regulation of TRP channels. Detailed results of GO and KEGG are placed in Supplementary Table 5.

On the other hand, we also performed GO and KEGG analysis of DEGs using ClueGO and the results were presets as Figure 3B. Results followed cut-off threshold P-value < 0.01 were just enriched in BP, CC and KEGG (Figure 3C). The BP ontology included keratinization (20%), antimicrobial humoral response (18%), regulation of epidermis development (12%), establishment of skin barrier (8%), cornification (6%) and hemidesmosome assembly (2%). The CC ontology included integrator complex (10%), intermediate filament (16%), and desmosome (2%). The KEGG pathway was Inflammatory mediator regulation of TRP channels (6%). The top 10 nodes ranked by MCC of cytoHubba

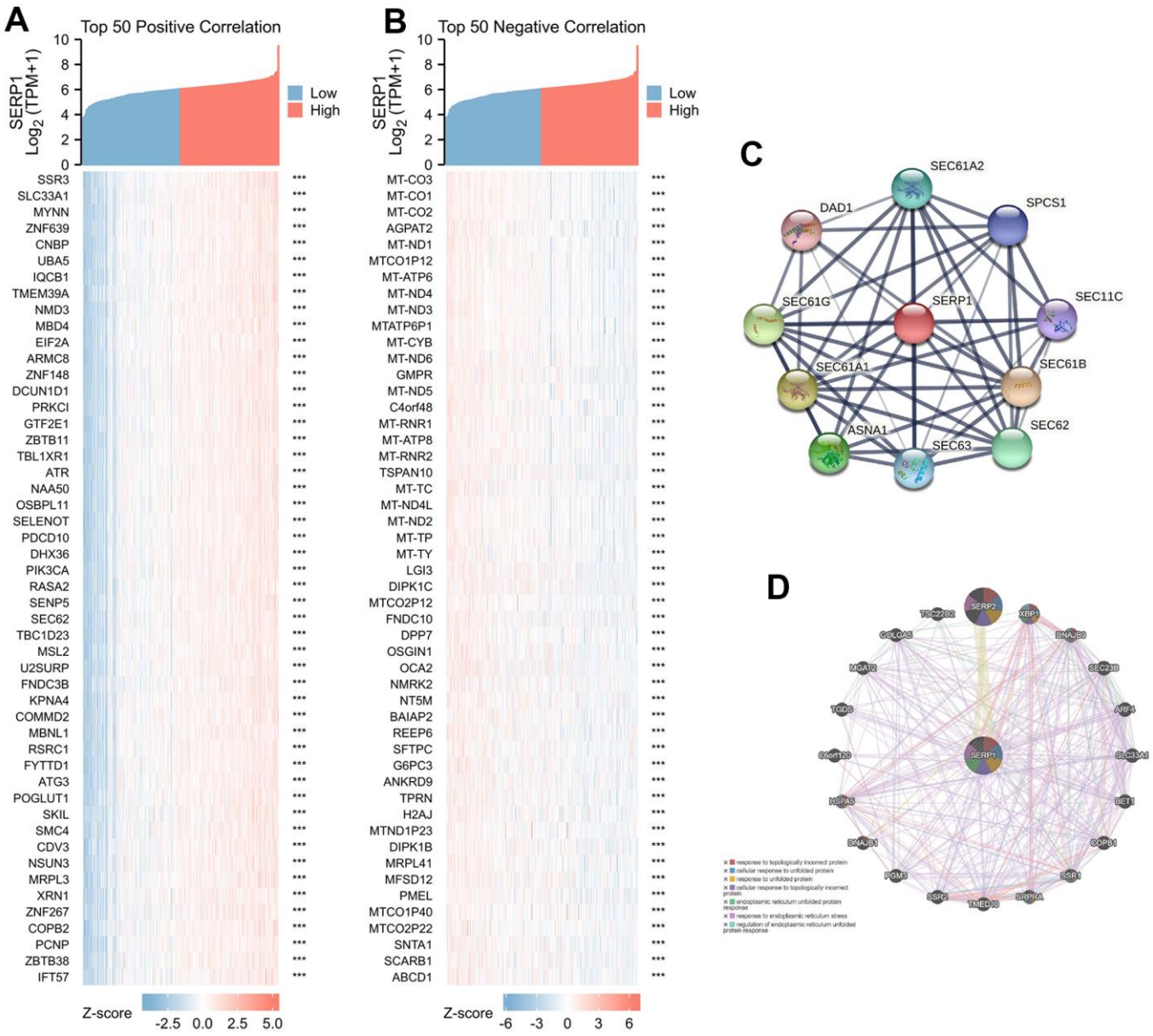

Figure 2. Genes related to SERP1. (**A**) The heatmap of top 50 positively RNAs related to SERP1, (**B**) The heatmap of top 50 negative RNAs related to SERP1, (**C**) The PPI network of SERP1 built via STRING, (**D**) The GGI network of SERP1 built via GeneMANIA.

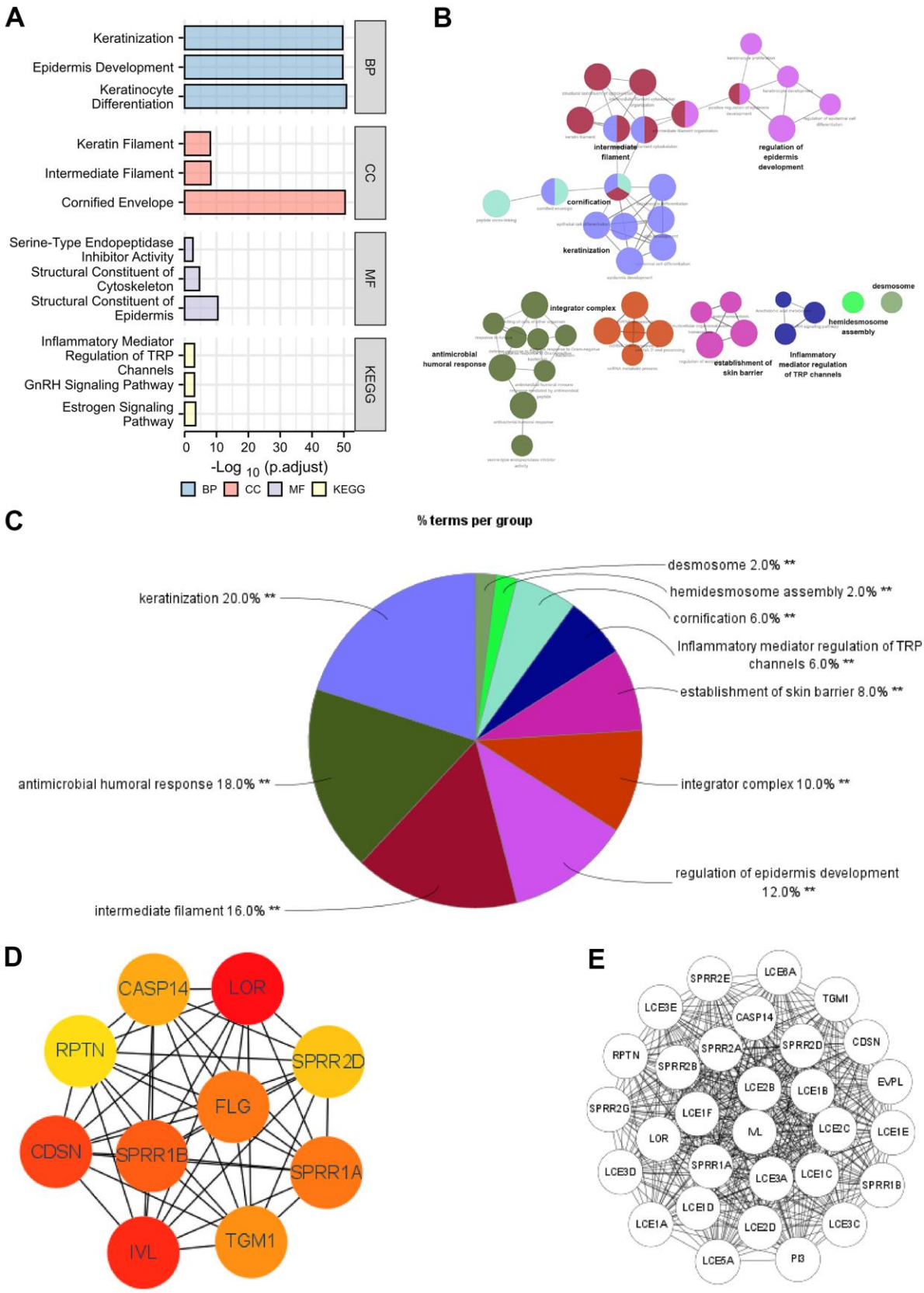

Figure 3. Functional enrichment analysis of DEGs between high and low expression of SERP1 in SKCM patients. (**A**–**C**) GO and KEGG pathway enrichment analyses for DEGs between High and -Low expression of SERP1 in SKCM patients. (**D**) The top 10 hub genes ranked by MCC of cytoHubba, (**E**) The top 30 hub genes ranked by MCODE.

were SPRR1B, CDSN, RPTN, IVL, SPRR2G, LOR, SPRR2E, EVPL, PI3 and TGM1 (Figure 3D) and modules with MCODE score = 30 including LCE1C, SPRR2A, LCE3E, LCE3D, LCE2D, LCE2C, LCE1E, SPRR1A, SPRR2D, SPRR2B, SPRR2E, SPRR2G, LCE1B, LCE3A, LCE1A, LCE3C, LCE5A, RPTN, LCE1D, SPRR1B, PI3, LOR, IVL, CASP14, LCE6A, CDSN, TGM1, LCE2B, LCE1F, EVPL were present in Figure 3E.

Gene set enrichment analyses (GSEA) identifies DEGs between high- and low-SERP1 expression related signaling pathways

We additionally conducted GSEA to identify signaling pathways that were differentially activated between high- and low-SERP1 expression groups in SKCM. The results indicated the Top 15 pathways were associated with Reactome GPCR ligand binding, Reactome G alpha i signaling events, Reactome class a1 rhodopsin like receptors, Reactome leishmania infection, Naba core matrisome, Kegg neuroactive ligand receptor interaction, Kegg cytokine-cytokine receptor interaction, Wp GPCRs class a rhodopsin-like, Reactome anti-inflammatory response favouring leishmania parasite infection, Reactome G alpha q signaling events, Kegg chemokine signaling pathway, Reactome peptide ligand binding receptors, Reactome cell surface interactions at the vascular wall, Reactome FC epsilon receptor (FCERI) signaling and Reactome immunoregulatory interactions between a lymphoid and a nonlymphoid cell (Figure 4A–4O). We further list the Top 50 pathways in Supplementary Table 6.

Genetic alteration and protein localization of SERP1 in SKCM patients

We analyzed the genetic mutations of SERP1 expression in SKCM patients via the cBioPortal online tool. Based on TCGA, SERP1 mutated lowly in SKCM via pan-cancers analysis (Figure 5A). Figure 5B showed the mutation rate of SERP1 genes was 1.1%. Figure 5C indicated the major form alterations of the SERP1 genes in SKCM is amplification. Figure 5D presented there was an overall higher amount of amplification occurrence of SERP1 mRNA expression in SKCM patients. We further explored the correlation between genetic mutations and the prognosis of SKCM patients. The statistically significant result was showed in Figure 5E that SERP1 genetic mutations decrease overall survival (OS) of SKCM patients ($p = 0.0402$). However, it is noteworthy that the expression of SERP1 protein did not show significant differences between tumor tissues and normal tissues in the Human Protein Atlas (Figure 5F).

Correlations between SERP1 and prognosis in SKCM patients

In order to explore the prognosis value of SERP1 in SKCM patients, we performed Kaplan-Meier analysis to evaluate its value on prediction of SKCM on clinical outcomes. The result found that OS (hazard ratio (HR): 0.62, 95% confidence interval (CI): 0.48–0.82, $p = 0.001$ (Figure 6A), disease specific survival (DSS) (HR: 0.65, 95% CI 0.49-0.87, $p = 0.004$) (Figure 6B) and progress free interval (PFI) (HR: 0.77, 95% CI 0.61-0.96, $p = 0.023$) (Figure 6C) for high SERP1 groups were all statistically better than those for the low SERP1 groups. In addition, in order to comprehensive understand the multidimensional prospect of the correlation of SERP1 expression to SKCM patient's survival, subgroup Kaplan-Meier analysis of OS was also performed according to different clinical variables. As Figure 6D–6O revealed, low SERP1 expression was significantly associated with worse OS in SKCM patients both of gender, female ($p = 0.009$) or male ($p = 0.006$), race white ($p = 0.001$), age ≤ 60 ($p = 0.005$), T stage is T2-4 ($p = 0.001$), N stage is N1-3 ($p = 0.008$), M stage is 0 ($p = 0.006$), Pathologic Stage is II-IV ($p = 0.007$), without radiation therapy ($p = 0.001$), Tumor tissue site is on the trunk ($p = 0.003$), having melanoma ulceration ($p = 0.003$) and Melanoma Clark Level Stage is II-V ($p = 0.012$). However, the high or low SERP1 expression on other cases did not show statistical significance on OS (Supplementary Figure 1A–1L).

Univariate Cox analysis showed that SERP1 expression was an independent risk factor for OS (HR: 0.591, 95% CI 0.405-0.861, $p = 0.006$) (Figure 7A and Supplementary Table 7) and DSS (HR: 0.584, 95% CI 0.395-0.864, $p = 0.007$) (Figure 7B and Supplementary Table 8). However, SERP1 expression did not have significant predictive power for PFI (HR: 0.769, 95% CI 0.555-1.067, $p = 0.116$) (Supplementary Table 9). Also available as independent risk factors through Univariate Cox analysis were N stage (HR: 2.945, 95% CI 1.887-4.597, $p < 0.001$), Melanoma ulceration (HR: 1.645, 95% CI 1.062-2.546, $p = 0.026$) and Breslow depth (HR: 2.028, 95% CI 1.312-3.136, $p = 0.001$) for OS and N stage (HR: 2.893, 95% CI 1.813-4.616, $p < 0.001$), Melanoma ulceration (HR: 1.709, 95% CI 1.087-2.687, $p = 0.020$) and Breslow depth (HR: 1.719, 95% CI 1.096-2.695, $p = 0.018$) for DSS, respectively.

The nomograms predicting OS (Figure 8A) and DSS (Figure 8B) was constructed based on significant risk factors identified from in the univariate Cox analyses. The score of the corresponding risk factors should be first identified by the point scale at the top of the nomogram. All scores were then summed and the corresponding 1-year, 3-year, and 5-year survival

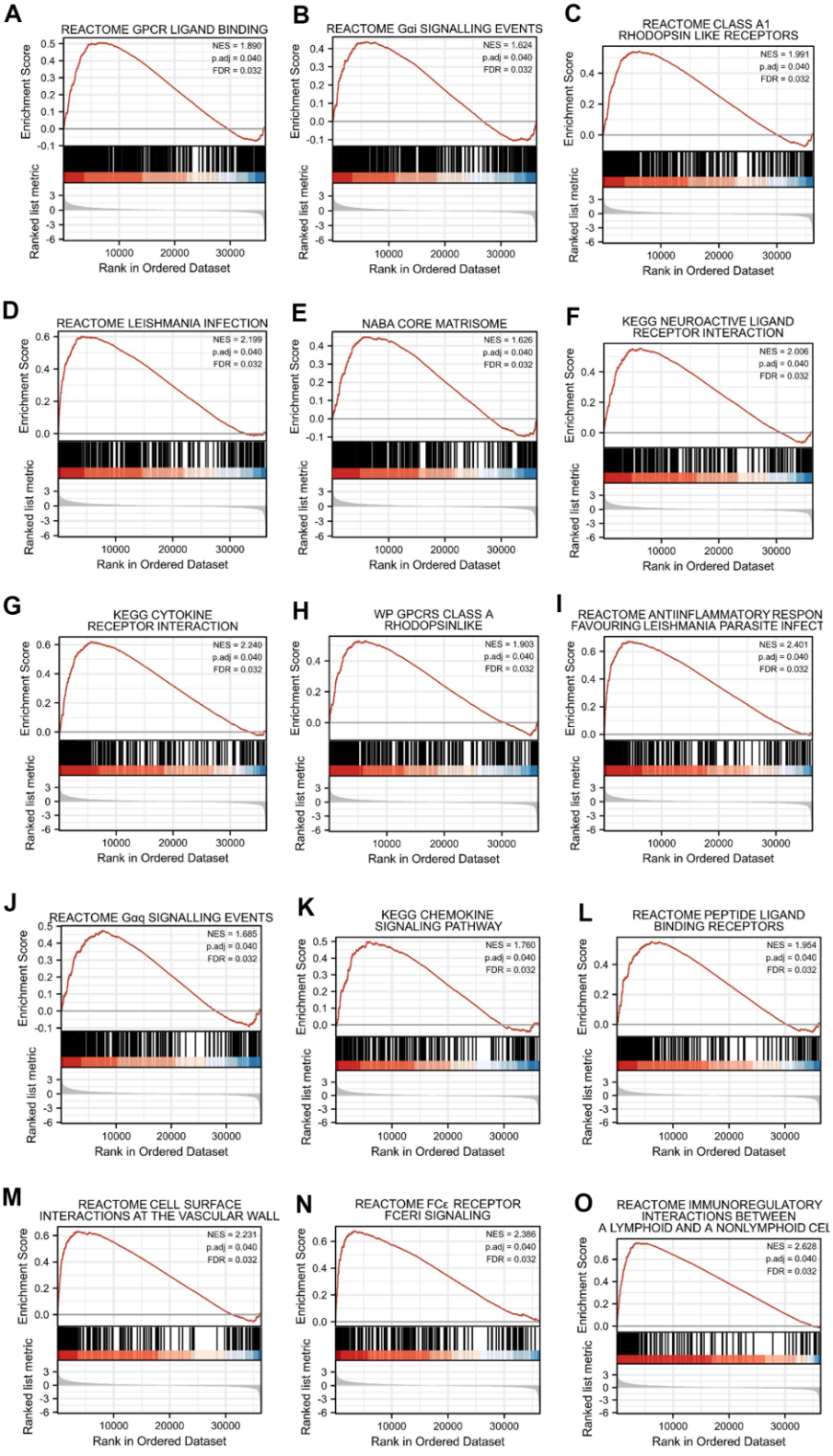

Figure 4. Top 15 enrichment plots of GSEA. The GSEA results showed that DEGs involved in (**A**) Reactome GPCR ligand binding, (**B**) Reactome G alpha i signalling events, (**C**) Reactome class a1 rhodopsin like receptors, (**D**) Reactome leishmania infection, (**E**) Naba core matrisome, (**F**) Kegg neuroactive ligand receptor interaction, (**G**) Kegg cytokine receptor interaction, (**H**) Wp GPCRs class a rhodopsin-like, (**I**) Reactome anti-inflammatory response favouring leishmania parasite infection, (**J**) Reactome G alpha q signalling events, (**K**) Kegg chemokine signaling pathway, (**L**) Reactome peptide ligand binding receptors, (**M**) Reactome cell surface interactions at the vascular wall, (**N**) Reactome FC epsilon receptor (FCERI) signaling and (**O**) Reactome immunoregulatory interactions between a lymphoid and a nonlymphoid cell.

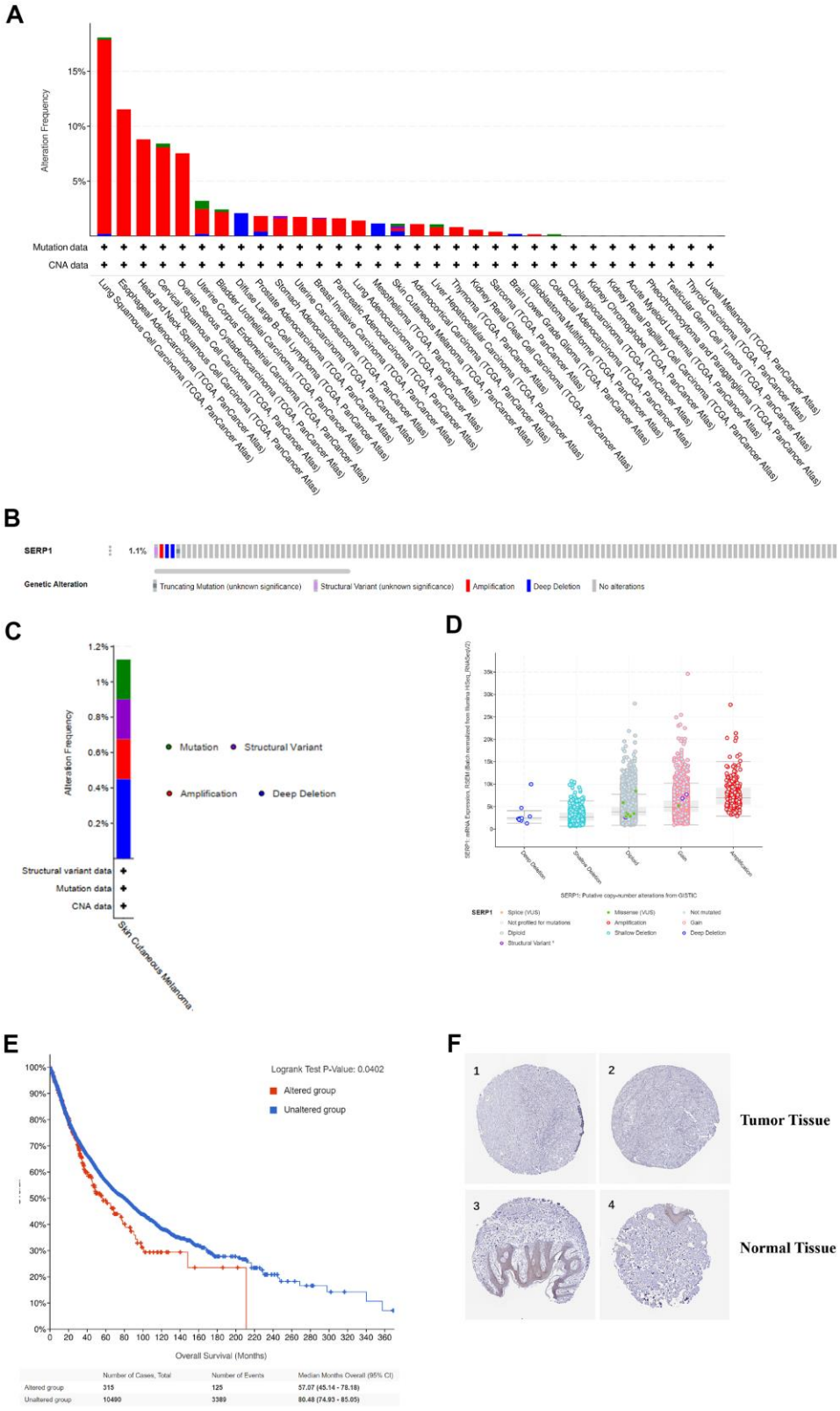

Figure 5. Genetic alteration and protein localization of SERP1 in SKCM patients. (**A**) Bar chart of SERP1 mutation in pan-cancers based on TCGA database. (**B**) SERP1 gene expression and mutation analysis in SKCM. (**C**) The distribution of SERP1 genomic alterations in SKCM. (**D**) The graph of the correlation between SERP1 expression and copy number alterations in SKCM. (**E**) Kaplan-Meier curve of OS in SKCM patients with altered (red) and unaltered (blue) mRNA expression of the SERP1 gene. (**F**) The representative IHC staining images from HPA database presents SERP1 expression in normal and tumor tissues.

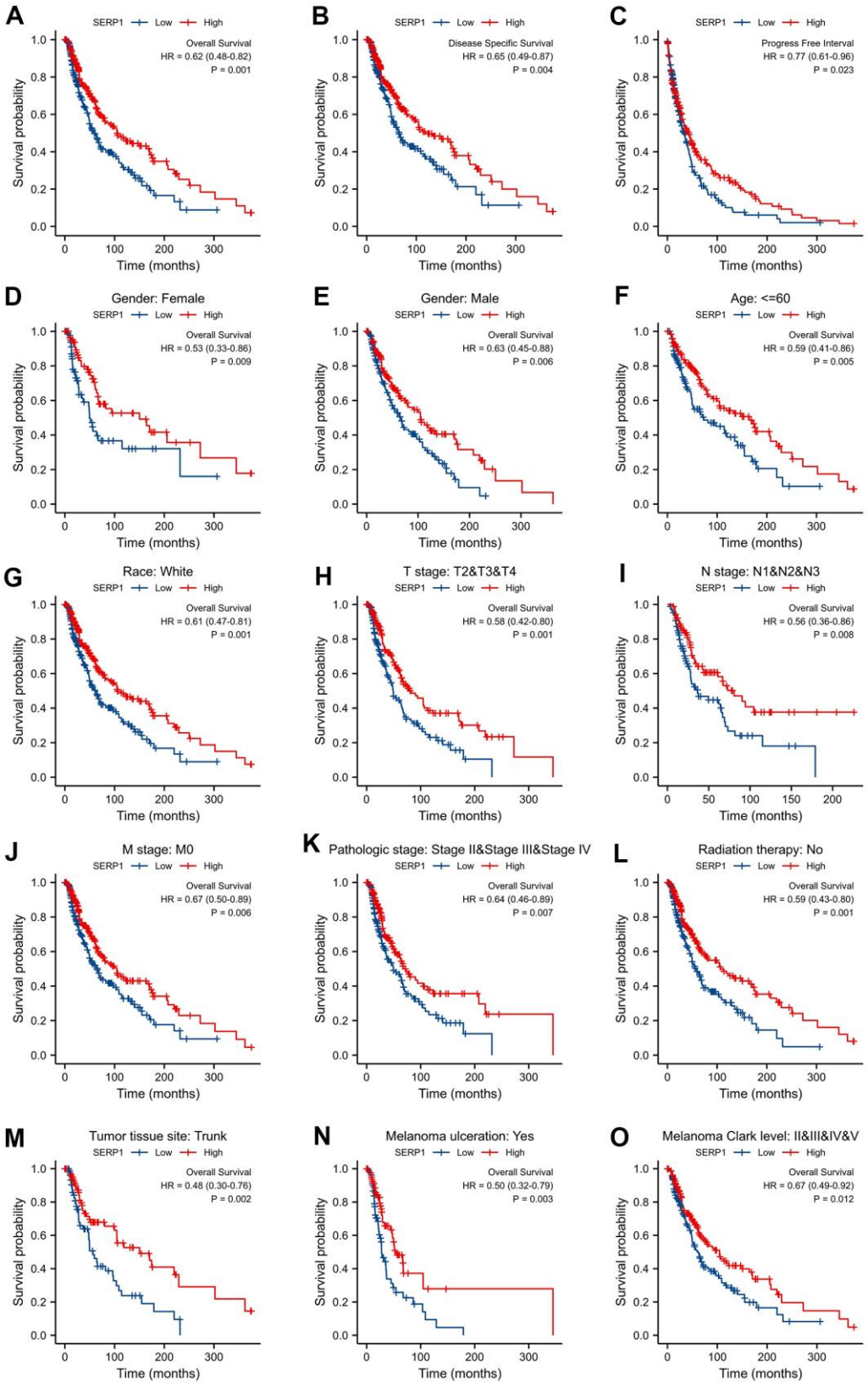

Figure 6. Correlations between SERP1 and prognosis in SKCM patients. (**A**) OS Kaplan-Meier curve for SERP1 in SKCM patients, (**B**) DSS Kaplan-Meier curve, (**C**) PFI survival Kaplan-Meier curve, (**D**–**O**) OS Kaplan-Meier curve of statistically significant subgroups for (**D**) Female, (**E**) male, (**F**) Age≤60, (**G**) Race white, (**H**) T stage (T2-T4), (**I**) N stage (N1-N3), (**J**) M stage (M0), (**K**) Pathologic Stage (Stage II-IV), (**L**) Radiation therapy No, (**M**) Tumor tissue site Trunk, (**N**) Melanoma ulceration Yes, (**O**) Melanoma Clark Level (Stage II-V).

probability was obtained corresponding to the scales at the bottom of the nomogram. The time-dependent ROC curves of OS and DSS are shown in Figure 8C, 8D, respectively. The 1, 3, and 5-year AUCs of OS are 0.404 (95% CI 0.308-0.500), 0.388 (95% CI 0.324-0.453) and 0.398 (95% CI 0.334-0.463), respectively. The 1, 3, and 5-year AUCs of DSS are 0.432 (95% CI 0.324-0.539), 0.401 (95% CI 0.333-0.469) and 0.408 (95% CI 0.341-0.475), respectively. These suggesting that low SERP1 has a good predictive efficacy and diagnostic accuracy for survival of SKCM patients. Details of the ROC information are presented in Supplementary Table 10.

Calibration curves showed a strong consistency between the possibilities obtained by the nomogram and the real results of 1-year and good consistency of 3 and 5-year for OS (Figure 8E) and DSS (Figure 8F). Furthermore, the C-index values were 0.686 (95% CI 0.662 to 0.710) and 0.664 (95% CI 0.638 to 0.690) for OS and DSS, respectively. These revealed the good credibility of the nomogram.

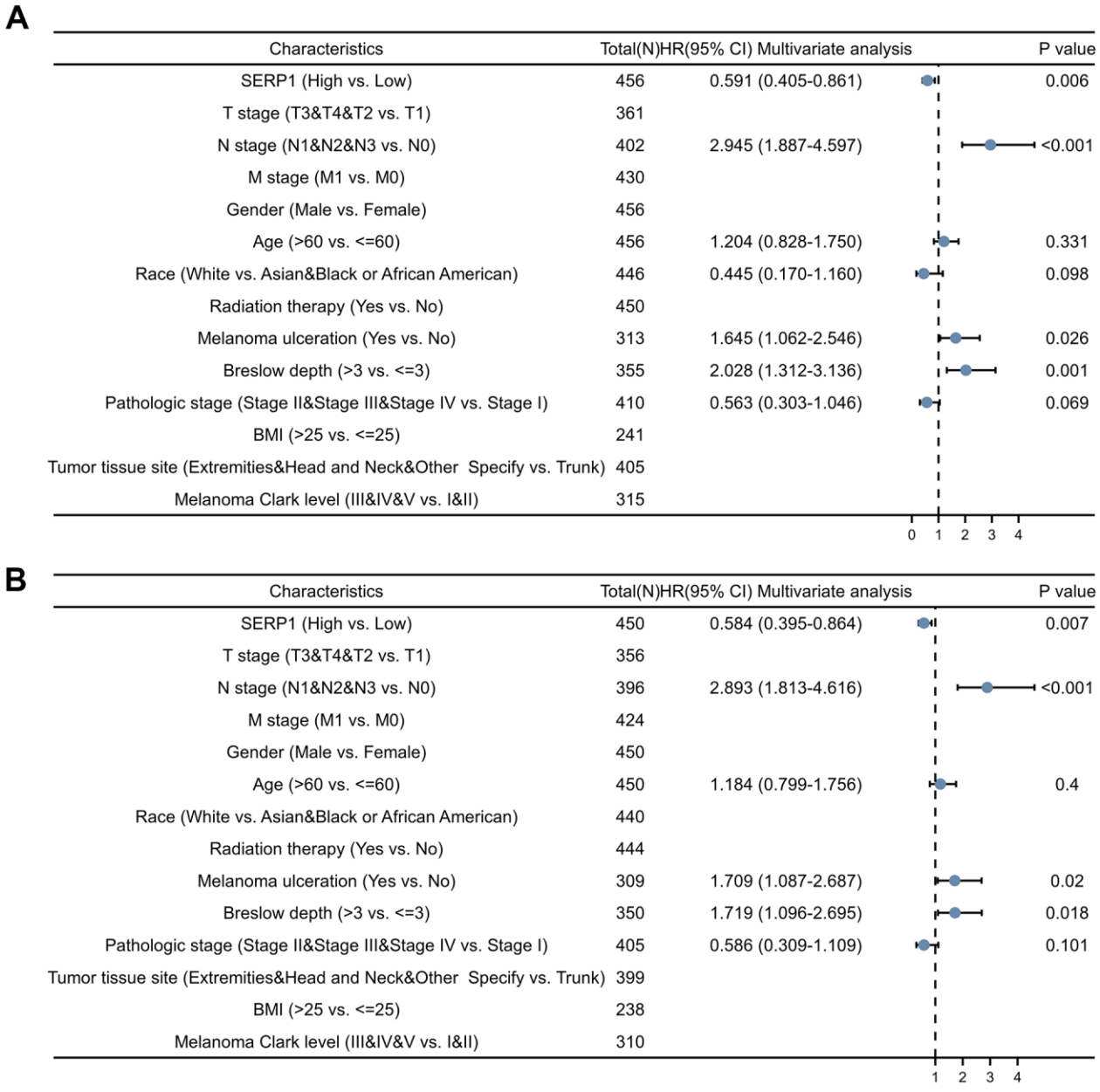

Figure 7. Forest plots of different clinical variables for SERP1 in SKCM patients. Forest plot of different clinical variables on OS (**A**) and DSS (**B**) by multivariate cox regression analysis.

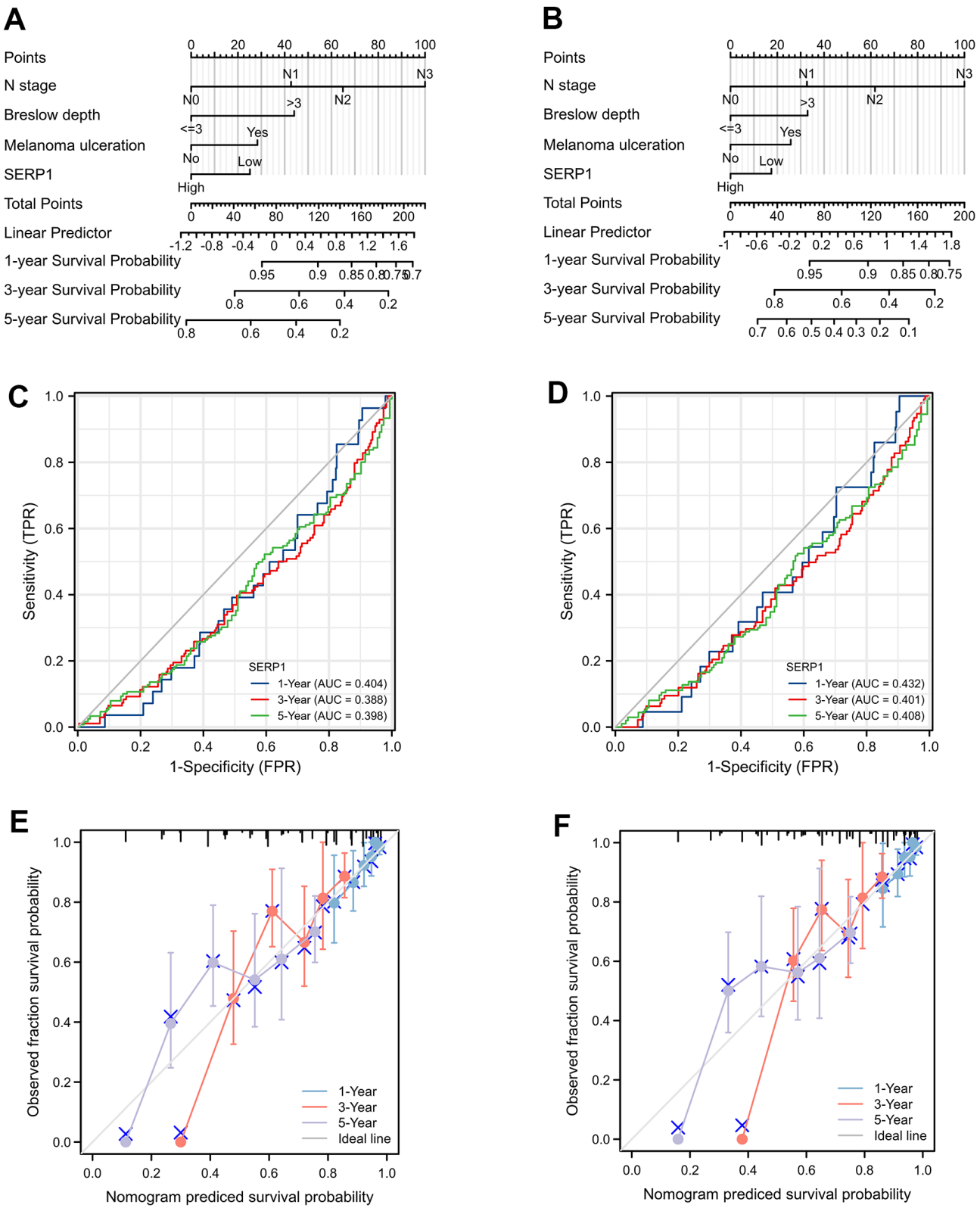

Figure 8. The prognostic nomogram for predicting OS and DSS probability. (**A**) The prognostic nomogram for predicting OS probability by the multivariable Cox regression model via the four statistically significant predictors, such as SERP1, N stage, Melanoma ulceration and Breslow depth. (**B**) The prognostic nomogram for predicting DSS probability. (**C**) The time-dependent ROC curves of OS for 1, 3, 5 year. (**D**) The time-dependent ROC curves of DSS for 1, 3, 5 year. (**E**) The calibration curve of OS for 1, 3, 5 year. (**F**) The calibration curve of DSS for 1, 3, 5 year.

Correlation between SERP1 expression and clinical variables in SKCM patients

The Figure 9A–9E revealed the correlation between the expression of SERP1 and the different clinical variables of SKCM patients. SERP1 expression level significantly related to the T stage ($p = 0.037$), Pathologic stage ($p = 0.009$), Radiation therapy ($p = 0.013$), Breslow depth ($p < 0.001$) and Melanoma ulceration ($p = 0.043$). The higher SERP1 expression was associated with lower T stage and Pathologic stage, shallower Breslow depth and fewer ulceration. Moreover, the expression of SERP1 was lower in patients with radiation therapy experience.

Correlation of SERP1 and immune cell infiltration in SKCM

The results found that T helper cells ($r = 0.510$, $p < 0.001$), Tcm ($r = 0.340$, $p < 0.001$), Tgd ($r = 0.330$, $p < 0.001$), Th2 cells ($r = 0.230$, $p < 0.001$), Macrophages ($r = 0.200$, $p < 0.001$), Th1 cells ($r = 0.190$, $p < 0.001$), B cells ($r = 0.180$, $p < 0.001$), aDC ($r = 0.140$, $p = 0.002$), T cells ($r = 0.140$, $p = 0.003$), CD8 T cells ($r = 0.130$, $p = 0.004$), Eosinophils ($r = 0.130$, $p = 0.005$) showed positive association with SERP1 expression. The NK CD56 bright cells ($r = -0.210$, $p < 0.001$), NK cells ($r = -0.180$, $p < 0.001$), Mast cells ($r = -0.170$, $p < 0.001$), pDC ($r = -0.130$, $p = 0.005$), Th17 ($r = -0.097$, $p = 0.036$) were negatively correlated with SERP1 (Figures 10A, 11). However, the TFH, Cytotoxic cells, iDC, NK CD56dim cells, Neutrophils, Tem, DC, Treg showed no significant correlation with SERP1 (Supplementary Figure 2).

We further evaluated the infiltration levels of 24 immune cells above in high- or low-SERP1 expression group (Figure 10B). The result indicated in high SERP1 expression group, the T cells ($p = 0.018$), aDC ($p = 0.009$), B cells ($p < 0.001$), Eosinophils ($p = 0.027$), Macrophages ($p < 0.001$), T helper cells ($p < 0.001$), Tcm ($p < 0.001$), Tgd ($p < 0.001$), Th1 cell ($p < 0.001$), Th2 cells ($p < 0.001$) infiltration levels were significantly higher than those in low SERP1 expression group. But the NK CD56 bright cells ($p < 0.001$), NK cells ($p = 0.016$), Mast cells ($p = 0.003$) infiltration level was significant lower in high SERP1 expression group than low SERP1 expression group.

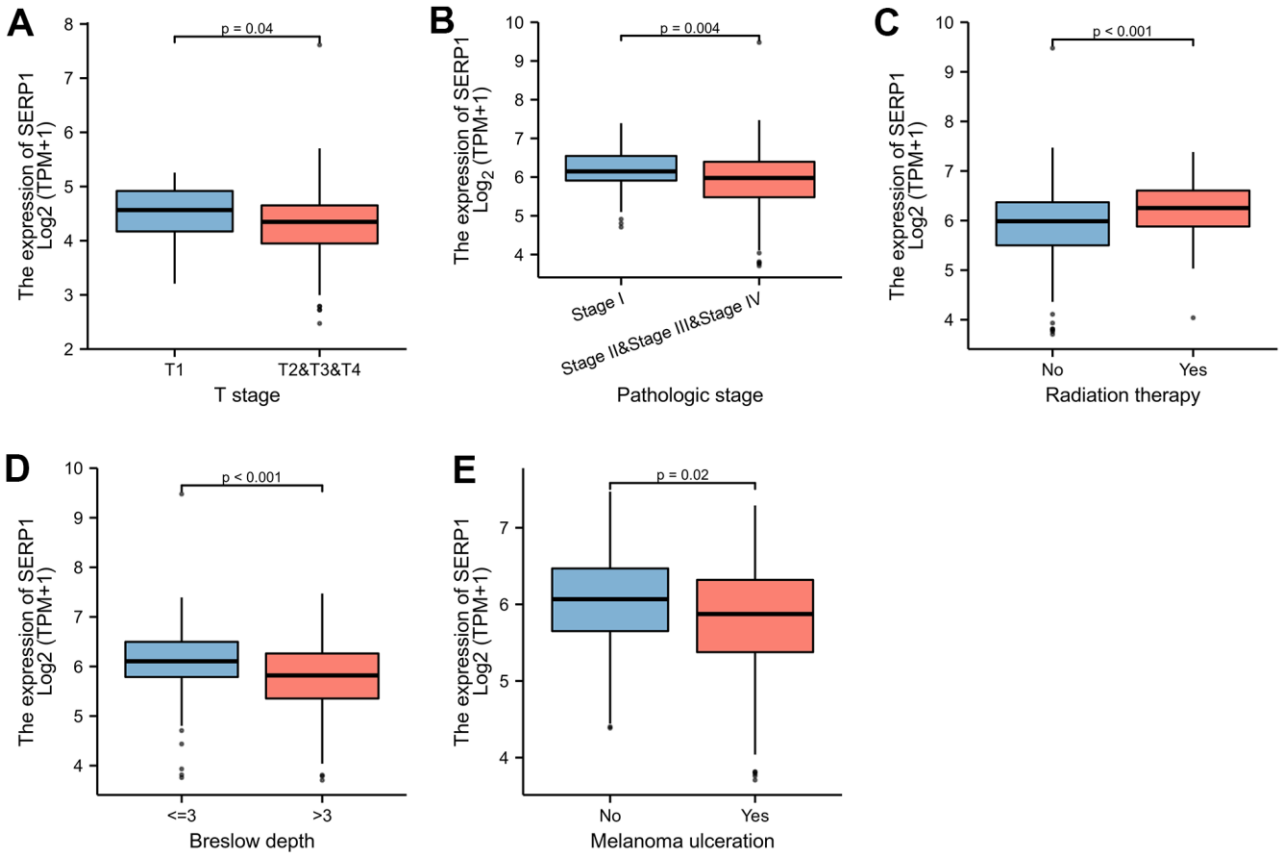

Figure 9. SERP1 expression is associated with different clinical variables in SKCM patients. (**A**) T classification, (**B**) Pathologic stage, (**C**) Radiation therapy, (**D**) Breslow depth, (**E**) Melanoma ulceration.

Moreover, the association of SERP1 expression with markers of mainly relative immune infiltration cells and immune checkpoints of SKCM were explored. The former mainly included.

CD8+ T cell, monocyte, tumor-associated macrophage (TAM), M1 macrophage, M2 macrophage, neutrophils, DC, Th1, Th2, Tfh, Th17 and Treg. The latter mainly included PD-1, PD-L1, CTLA4, LAG3, TIM-3, GZMB, TIGIT and BTLA. We found that SERP1 expression is significantly positive correlated with the most (36 of 41) immune cell markers in SKCM ($P < 0.05$) (Table 2). Although none of the correlations were high ($r < 0.5$), but it suggests that SERP1 is extensively involved in regulating immune cell function in SKCM. It is worth noting that, SERP1 expression was positively correlated with numerous immune checkpoint gene markers, including PD-1, PD-L1, PD-L2, CTLA4, LAG3, TIM-3, GZMB, TIGIT (Table 3). These results suggest that SERP1 expression may be associated with the immunotherapeutic effect of SKCM.

DISCUSSION

The ER is a prominent organelle in eukaryotic cells that is involved in regulating calcium homeostasis, the

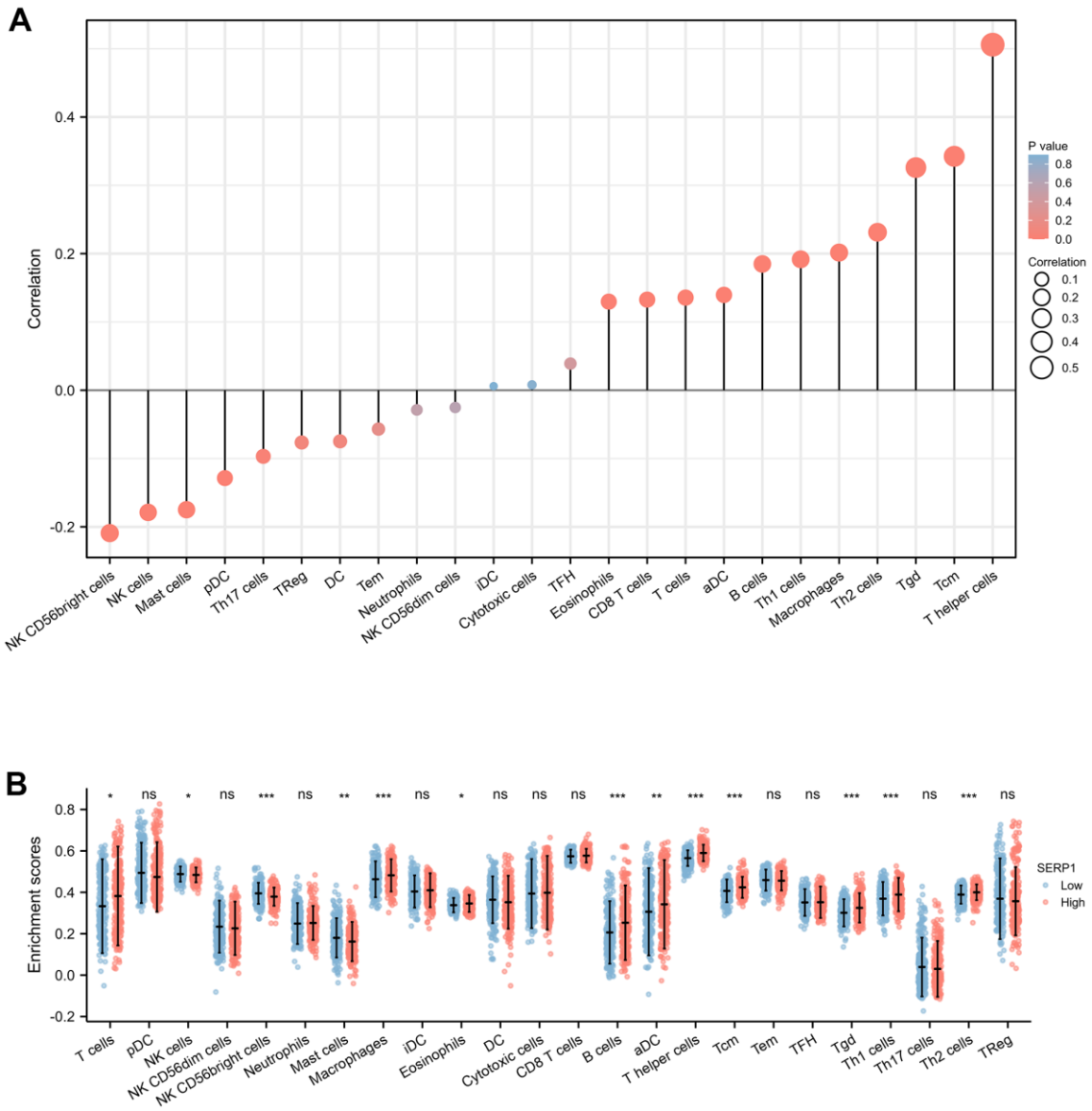

Figure 10. Associations of SERP1 expression and immune infiltration level in SKCM patients. (**A**) Correlation of SERP1 expression with immune infiltration level of 24 immune cell types by Spearman's analysis. (**B**) Twenty-four types of immune cells are plotted according to different SERP1 expression levels.* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

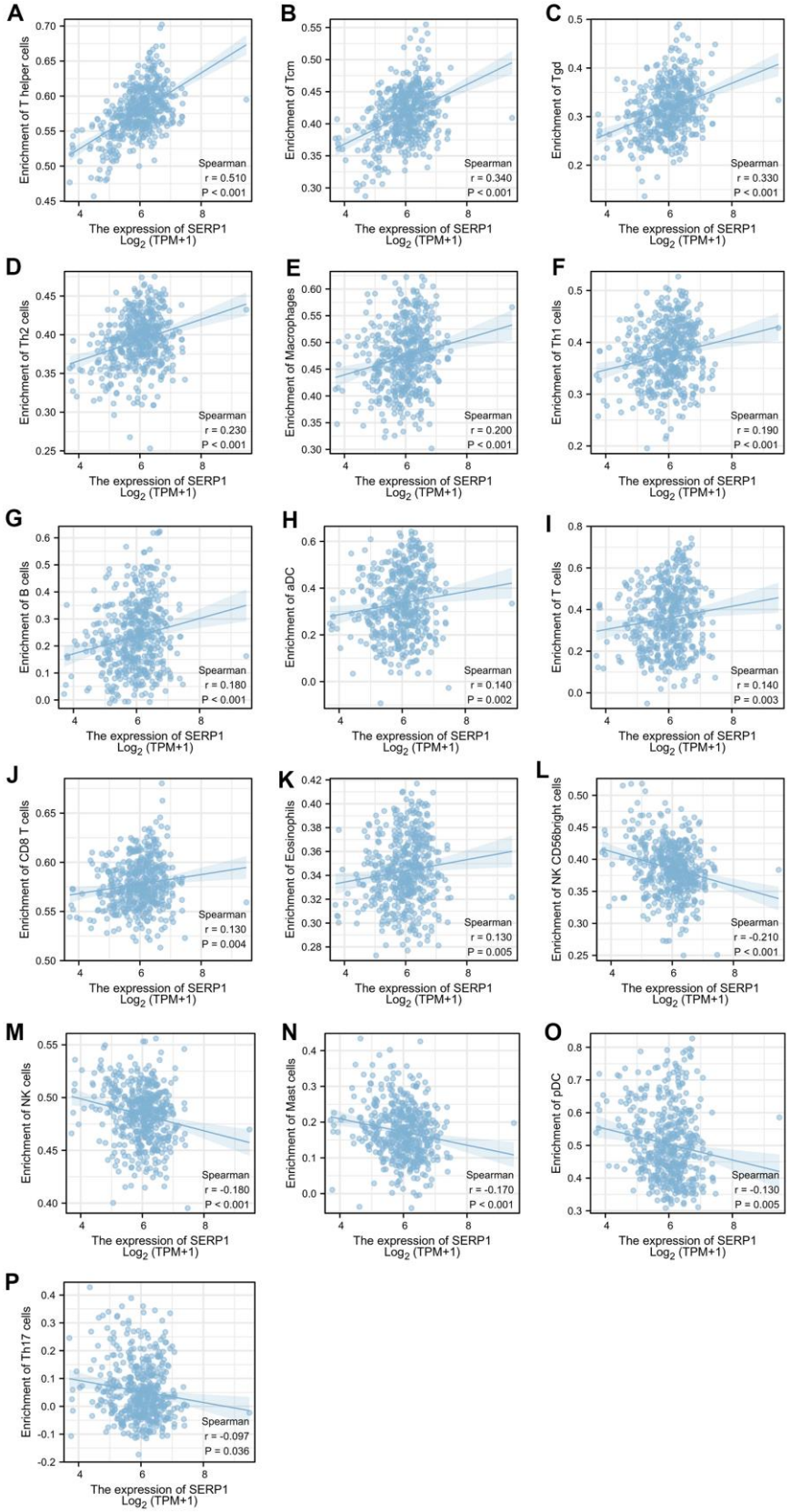

Figure 11. Relationship of SERP1 expression with immune cell level in SKCM. (**A**–**P**) SERP1 expression showed significant positive related to infiltrating levels of T helper cells, Tcm, Tgd, Th2 cells, Macrophages, Th1 cells, B cells, aDC, T cells, CD8 T cells, Eosinophils and significant negative related to infiltrating levels of NK CD56 bright cells, NK cells, Mast cells, pDC and Th17 cells.

Table 2. Correlation analysis between SERP1 and relate gene markers of immune cells in SKCM.

Immune cell	Biomarker	SERP1	
		R	*P*
CD8+ T cell	CD8A	0.200	<0.001
	CD8B	0.170	<0.001
Monocyte	CD115 (CSF1R)	0.340	<0.001
	CD14	0.240	<0.001
	CD86	0.390	0.001
TAM	CCL2	0.031	<0.001
	CD68	0.043	0.357
	IL10	0.350	<0.001
M1 macrophage	NOS2	0.043	0.351
	IRF5	0.180	<0.001
	PTGS2	0.240	<0.001
M2 macrophage	CD163	0.400	<0.001
	VSIG4	0.320	<0.001
	MS4A4A	0.380	<0.001
Neutrophils	CEACAM8	0.190	<0.001
	CD11b (ITGAM)	0.330	<0.001
	CCR7	0.170	<0.001
Dendritic cell	HLA-DPB1	0.150	<0.001
	HLA-DQB1	0.150	<0.001
	HLA-DRA	0.220	<0.001
	HLA-DPA1	0.150	<0.001
	BDCA-1 (CD1C)	0.230	<0.001
	BDCA-4 (NRP-1)	0.460	<0.001
	CD11C (ITGAX)	0.170	<0.001
Th1	T-bet (TBX21)	0.220	<0.001
	STAT4	0.370	<0.001
	STAT1	0.400	<0.001
	IFN-γ (IFNG)	0.250	<0.001
	TNF-α (TNF)	0.170	<0.001
Th2	GATA3	0.180	<0.001
	STAT6	0.080	0.082
	STAT5A	0.001	0.987
	IL13	0.130	0.005
Tfh	BCL6	0.390	<0.001
	IL21	0.350	<0.001
Th17	STAT3	0.300	<0.001
	IL17A	-0.110	0.019
Treg	FOXP3	0.130	0.004
	CCR8	0.340	<0.001
	STAT5B	0.250	<0.001
	TGFβ (TGFB1)	0.220	<0.001

Abbreviations: SERP1, Stress associated endoplasmic reticulum protein 1; SKCM, Skin Cutaneous Melanoma; TAM, tumor-associated macrophage, TAM.

Table 3. Correlation analysis between SERP1 and immune checkpoints in SKCM.

Immune checkpoints	SERP1	
	R	*P*
PD-1 (PDCD1)	0.130	<0.001
PD-L1(CD274)	0.320	<0.001
PD-L2(PDCD1LG2)	0.390	<0.001
CTLA4	0.230	<0.001
LAG3	0.170	<0.001
TIM-3 (HAVCR2)	0.310	<0.001
GZMB	0.180	<0.001
TIGIT	0.260	<0.001
BTLA	0.310	<0.001

Abbreviations: SERP1, Stress associated endoplasmic reticulum protein 1; SKCM, Skin Cutaneous Melanoma.

synthesis and folding of secretory and transmembrane proteins, and lipid biosynthesis [14]. Disruption of protein folding in the ER can result from different types of stress, such as inflammatory stimuli, a disruption of calcium homeostasis, nutrient deprivation, an imbalance in redox homeostasis, or an acute increase in protein synthesis [15]. Adverse environmental conditions caused by tumors, such as high metabolic demand, hypoxia, nutrient deprivation, acidosis, and accumulation of reactive oxygen species, can lead to ER stress [16]. This triggers an adaptive response called the unfolded protein response (UPR), aimed at increasing the folding and clearance capacity, thus restoring ER homeostasis [17]. Our understanding of the role of ER stress in various tumors has grown recently [18]. The ER stress response has emerged as a crucial player in initiation, metastasis, prognosis, and immunity in various tumors [19, 20] and is becoming a prevalent target in cancer therapy [21]. SKCM is a highly aggressive and fatal cancer with poor treatment outcomes after progression [22]. Although an association between SKCM and ER has been demonstrated [23], there are few studies of specific genetic markers.

SERP1 is a polypeptide produced during ER stress that protects unfolded proteins from degradation and is associated with poor prognosis in various tumors [12, 13, 24]. Since there had been no clinical studies or basic experiments investigating the effect of SERP1 on SKCM, we conducted a comprehensive bioinformatics exploration via large-scale bioinformatics databases. Our study revealed significantly lower SERP1 levels in patients with SKCM, identified DEGs with the greatest difference in expression between high- and low-SERP1 expression groups and identified transcripts that are highly correlated with SERP1 expression in SKCM. The PPI of SERP1 and GGI of its neighboring genes revealed that these genes are correlated with the response to ER stress and UPR regulation, suggesting that SERP1 participates in ER stress during SKCM tumorigenesis.

In this study, low SERP1 expression was associated with lower OS, PFI, and DSS rates. We also observed significant associations between low SERP1 expression and OS with several patient and clinical parameters including race, age, TNM stage, pathologic stage, absence of radiation therapy, tumor tissue site, melanoma ulceration, and Melanoma Clark Level. Low SERP1 expression correlated with several clinical variables, such as T stage, pathologic stage, Breslow depth, Melanoma ulceration, and radiation therapy. These results illustrate that SERP1 serves an important role in SKCM proliferation, metastasis, and treatment strategy.

The mutation rate of SERP1 in patients with SKCM in this study was 1.1%, with genetic alterations correlated with shorter OS; moreover, results indicated that SERP1 expression level is an independent prognostic factor of OS and DSS for patients with SKCM, suggesting that SERP1 is involved in SKCM progression. However, the results of this study differ from previous findings that high SERP1 expression is associated with poor tumor prognosis [12, 13, 24]. This discrepancy may be related to the dual effects of ER stress. Different levels of ER stress, cell types, and specific tumor microenvironments will lead to different outcomes of the ER stress response [25]. On one hand, excessive or unresolved ER stress can cause cell death. On the other hand, moderate non-lethal ER stress will lead to ER homeostasis recovery and adapt tumor cells to autophagy and apoptosis [26]. Low SERP1 expression in patients with SKCM may be a manifestation of a relatively moderate magnitude of ER stress, thus maintaining the survival of cutaneous melanoma cells.

Early stage SKCM has a better prognosis after surgical resection; however, SKCM tends to subcutaneously metastasize to regional lymph nodes and distant organs, which complicated treatment for advanced stages [27]. Netanely et al. [28] divided melanoma tumors into four subtypes according to different gene expression signatures and validated the classification. They found that the keratin group has the lowest survival rate, significantly higher Breslow depths, and higher pathologic T values. This group is characterized by overexpression of cornification, epidermis development, and keratin-related genes that play a crucial role in forming the outermost skin barrier [29]. This is consistent with the GO and KEGG enrichment analysis of SERP1 in patients with SKCM in our study. Additionally, the predicted genes used in the study to validate the keratin group, including IVL and SPRR1B [28], are consistent with the hub genes of DEGs from cytoHubba and MCODE in our analysis. Thus, keratinization, epidermis development, and cornification related to SERP1 are likely to play an important role in the tumorigenesis and prognosis of SKCM.

SKCM cancer cells and the tumor microenvironment (TME) constitute the SKCM tissue [30]. The TME of SKCM includes the surrounding immune cells, fibroblasts, inflammatory cells, various signaling molecules, and extracellular matrix. The immune cells within the TME and the way they are regulated play an important role in tumorigenesis and progression [31].

Tumor-infiltrating immune cells of the adaptive and innate immune systems infiltrate into the TME and serve a critical role in the modulation of tumor progression [32]. Our results revealed a positive association between SERP1 expression and the infiltration levels of most T cell populations, B cells, macrophages, aDCs, and eosinophils. Conversely, the infiltration levels of NK cells, mast cells, NK CD56 bright cells, pDC and Th17 were negatively correlated with SERP1 expression. Previous studies [33, 34] found that high infiltrates of B cell, T cells were related to a better survival in the SKCM, to some extent, is consistent with our findings.

Additionally, we observed significant differences between infiltrating immune cells in SKCM between the high- and low-SERP1 expression groups. Compared with the low-SERP1 expression group, most T cell populations, B cells, macrophages, aDCs, and eosinophils were present in significantly higher proportions in the high-SERP1 expression group while NK CD56 bright cells, NK cells and Mast cells were significantly lower. The above results agree with previous single-cell-based studies [35–38] of immune infiltrates in melanoma. T cells serve an important role in effective anti-tumor immunity owing to their potent tumor-killing ability [32]. The main function of T helper cells is to amplify the immune response of other immune cells, such as the killing effect of cytotoxic T cells. Th1 cells heighten antigen presentation and are key players in cellular immunity while Th2 cells promote B-cell maturation and enhance the humoral immune response [39]. B cells can perform anti-tumor immune functions by participating in humoral and cellular immunity and acting as antigen-presenting cells [40]. Macrophages and DCs contribute to intrinsic immunity and can regulate tumor progression [41, 42]. Diminished cytotoxic effects and altered expression of pro-inflammatory factors in the tumor microenvironment can impede NK cell function [43] and can lead to immune escape [44]. Mast cells are derived from bone marrow hematopoietic progenitor cells and are broadly distributed throughout the body. Early mast cell infiltration is widespread in solid tumors, especially in malignant melanoma [45]. Mast cells can release various proangiogenic factors, such as VEGF, FGF-2, PDGF, IL-6, tryptase, and chymase, to promote tumor angiogenesis and induce neovascularization. It can also release matrix metalloproteinases to promote tumor invasiveness [46]. Our results of immune infiltration combined with the above reveal that SERP1 is involved in both the innate and adaptive immune responses to SKCM. This, to some extent, implies that differences in immune infiltration produced by SERP1 expression profoundly affect the prognosis of patients with SKCM.

Moreover, our study found that SERP1 expression levels positively correlated with most immune cell markers suggesting the positive role of SERP1 in modulating tumor immunology in SKCM. Interestingly, the correlation between the gene markers of M1 macrophage and SERP1 expression was lower than that of M2 macrophage, implying SERP1 may be involved in the polarization of TAM. Further, our result confirmed that SERP1 expression in SKCM patients was positively correlated with numerous immune checkpoint gene markers. Given that multiple targeted immune checkpoint blockade therapies, especially PD-1 [47, 48] and CTLA4 [49, 50], have shown significant efficacy in melanoma treatment, SERP1 expression levels in SKCM patients may also influence the efficacy of immunotherapy.

In conclusion, the expression of SERP1, an ER response-related gene, is reduced in patients with SKCM. SERP1 expression level is associated with diverse clinical variables. Decreased SERP1 expression results in decreased survival of patients with SKCM with some clinical features and is an independent risk factor for poor prognosis in SKCM. Thus, it can serve as a biomarker with the capacity to predict prognosis in patients with SKCM. Genes closely associated with SERP1 are primarily involved in keratinization,

epidermis development, and cornification, which play a pivotal role in the poor prognosis and tumorigenesis of SKCM. SERP1 expression is also significantly associated with the infiltration of multiple immune cells and immune checkpoints, involved in both the innate and adaptive immune systems of SKCM, and participates in the construction of the SKCM TME. We hope that further study on SERP1 in SKCM will confirm its potential for clinical application. It is expected that further exploration of how SERP1 is involved in ER stress and affects the TME will shed light on immunotherapy options for SKCM.

MATERIALS AND METHODS

Gene expression and clinical data

We obtained the mRNA expression and clinical data from TCGA (https://cancergenome.nih.gov). The data for the corresponding 813 normal tissue samples were obtained from the GTEx (https://gtexportal.org/). Samples with "0" values for gene expression or with inadequate prognostic information were excluded.

RNA-sequencing data in Fragments Per Kilobase per Million format (FPKM) were converted and normalized by the Toil [51] process as transcripts per million (PTM) reads and log2 transformed for further analysis. The clinical characteristics of the 472 patients included in the study are summarized in Supplementary Table 1. Location and qualitative data of SERP1 protein in SKCM and normal tissues were evaluated by the Human Protein Atlas (https://www.proteinatlas.org/) [52].

DEGs analysis between SKCM patients with high and low SERP1 expression

We performed a "DESeq2" analysis [53] in R to identify DEGs between SERP1-high and SERP1-low SKCM patients identified by unpaired Student's t-test. Thresholds were set as an adjusted $P < 0.05$ and absolute log-fold change > 3. Identified genes were analyzed and presented as volcano plots. The top 15 up- or down-regulated genes were presented as heat maps. All data visualization was achieved using the "ggplot2" package in R. We set a relatively high threshold (log-fold change > 3) with the aim of selecting the DEGs with the greatest degree of change associated with SERP1-high and SERP1-low expression. The top 15 DEGs were selected to further screen the genes most associated with SERP1-high and SERP1-low expression.

SERP1 correlation analysis in patients with SKCM

We performed correlation analysis between SERP1 and other RNAs in patients with SKCM using TCGA data via the "stat" package in R. Pearson correlation coefficients were calculated and the top 50 genes most positively and negatively associated with SERP1 were filtered to construct heat maps using the "ggplot2" package in R.

PPI network and GGI network analysis

To collect and integrate potential protein interactions with SERP1, we searched the STRING database (https://string-db.org/) [54] and conducted a PPI network analysis. A confidence score > 0.7 was set as the significance threshold. GeneMANIA (http://www.genemania.org) [55] uses extensive genomics and proteomics data to discover functionally similar genes. The database is used to generate hypotheses about gene function, analyze gene lists, and prioritize genes for functional analysis. We searched for SERP1 in this database to predict the gene–gene interaction (GGI) network.

Functional enrichment analysis of DEGs between patients with SKCM with high- and low-SERP1 expression

The "clusterProfiler" [56] and "org.Hs.eg.db" packages of R and the "ClueGO" app of Cytoscape (v3.8.2) were used to conduct GO function and KEGG pathway enrichment analyses for statistically significant DEGs. A p-value < 0.01 was set as the cut-off threshold for GO and KEGG pathway enrichment analyses. The results were presented as a bar plot via the "ggplot2" package in R. Additionally, we also imported the DEGs into the STRING database to build a PPI network map and completed the GO and KEGG signaling pathway enrichment analyses using the ClueGO plugin of Cytoscape. The MCODE and cytoHubba plugins were used to identify key modules. The top 10 nodes ranked by MCC of cytoHubba and modules with MCODE score = 30 were presented.

Gene set enrichment analysis

We performed GSEA via the "clusterProfiler" [56] package to determine the biological pathway differences between high- and low-SERP1 groups. Pathways with a false discovery rate (FDR) < 0.25 and an adjusted p-value < 0.05 were considered to be remarkably changed. Gene set permutation was performed 1,000 times for each analysis.

cBioPortal

We searched and downloaded mutation information and corresponding clinical data from cBioPortal (https://www.cbioportal.org/) [57], a comprehensive database that provides visual and multidimensional cancer genomics data.

Kaplan–Meier analysis

We conducted Kaplan–Meier analysis via the "survival" package to compare the OS, DSS, and PFI rates between the high- and low-SERP1 gene expression groups. Subgroup Kaplan–Meier analysis of OS was also performed based on different clinical variables. The p-value was determined by Cox regression analysis and the survival curves were visualized via the "survminer" package.

Correlation between SERP1 expression and immune infiltration in SKCM

We used the gene set variation analysis package [58] to investigate the correlation between the SERP1 expression and tumor-infiltrating immune cells in patients with SKCM. Twenty-four types of immune cells were included in the analysis: T cells, aDC (activated DC), B cells, CD8 T cells, cytotoxic cells, DC, eosinophils, iDC (immature DC), macrophages, mast cells, neutrophils, NK CD56 bright cells, NK CD56 dim cells, NK cells, pDC (plasmacytoid DC), T helper cells, Tcm (central memory T cells), Tem (effector memory T cells), Tfh (T follicular helper cells), Tgd (gamma delta T cells), Th1 cells, Th17 cells, Th2 cells, and Treg (regulatory T cells). The association of SERP1 with immune cell markers as well as immune checkpoints were also explored. Spearman's correlation was used to evaluate the correlation of gene expression with p-values < 0.05 considered statistically significant.

Statistical analysis

R software was used to perform statistical analyses in this study (version 3.6.3). The "ggplot2" package was used to present SERP1 gene expression as dot plots in patients with pan-cancer and SKCM. The median method of gene expression was selected for cutoff values. SERP1 expression in patients with SKCM with different clinical characteristics was analyzed using the Chi-squared test, Fisher test, and Wilcoxon rank sum test depending on the situation. The "survival" package was used to analyze the effect of SERP1 on survival with other clinical characteristics in SKCM patients via univariate and multivariate Cox regression. The predictive nomogram of 1-, 3-, and 5-year OS, DSS and PFI with SERP1 expression for SKCM patients and other relevant clinical parameters was constructed via a stepwise Cox regression model through the "rms" and "survival" packages. The ROC curve, C-index and calibration curve were used to verify the reliability of nomograms. ROC curves were analyzed and the areas under the ROC curve (AUC) were calculated via the timeROC (version 0.4) package of R software. The calibration curves were analyzed and plotted via rms (version 6.2-0) and survival (version 3.2-10) package of R software. The number of samples per group for our calibration curves is set to 40, the number of repetitions is 200, and the boot method is used.

Abbreviations

SKCM: Skin Cutaneous Melanoma; SERP1: Stress associated endoplasmic reticulum protein 1; ER: Endoplasmic reticulum; TCGA: The Cancer Genome Atlas; GTEx: Genotype-Tissue Expression; PPI: Protein-protein interaction; GGI: Gene–gene interaction; GO: Gene Ontology; KEGG: Genes and Genomes; GSEA: Gene set enrichment analyses; TPM: transcripts per million; FPKM: Fragments Per Kilobase per Million; DEGs: differentially expressed genes; OS: overall survival; DSS: disease specific survival; PFI: progress free interval; BP: biological process; MF: molecular function; CC: cellular component; HR: hazard ratio; CI: confidence interval; AUC: areas under the ROC curve; ROS: reactive oxygen species; UPR: unfolded protein response; TME: tumor microenvironment; ECM: extracellular matrix; MDSC: myeloid-derived suppressor cells; MMPs: matrix metalloproteinase; TAMs: tumor-associated macrophages; UPR: Unfolded protein response.

AUTHOR CONTRIBUTIONS

All authors contributed to the work presented in this paper. Yu designed the research and supervised the project. Fan performed statistical analysis of data and prepared all figures and tables. Fan and Liang wrote the manuscript. All authors reviewed and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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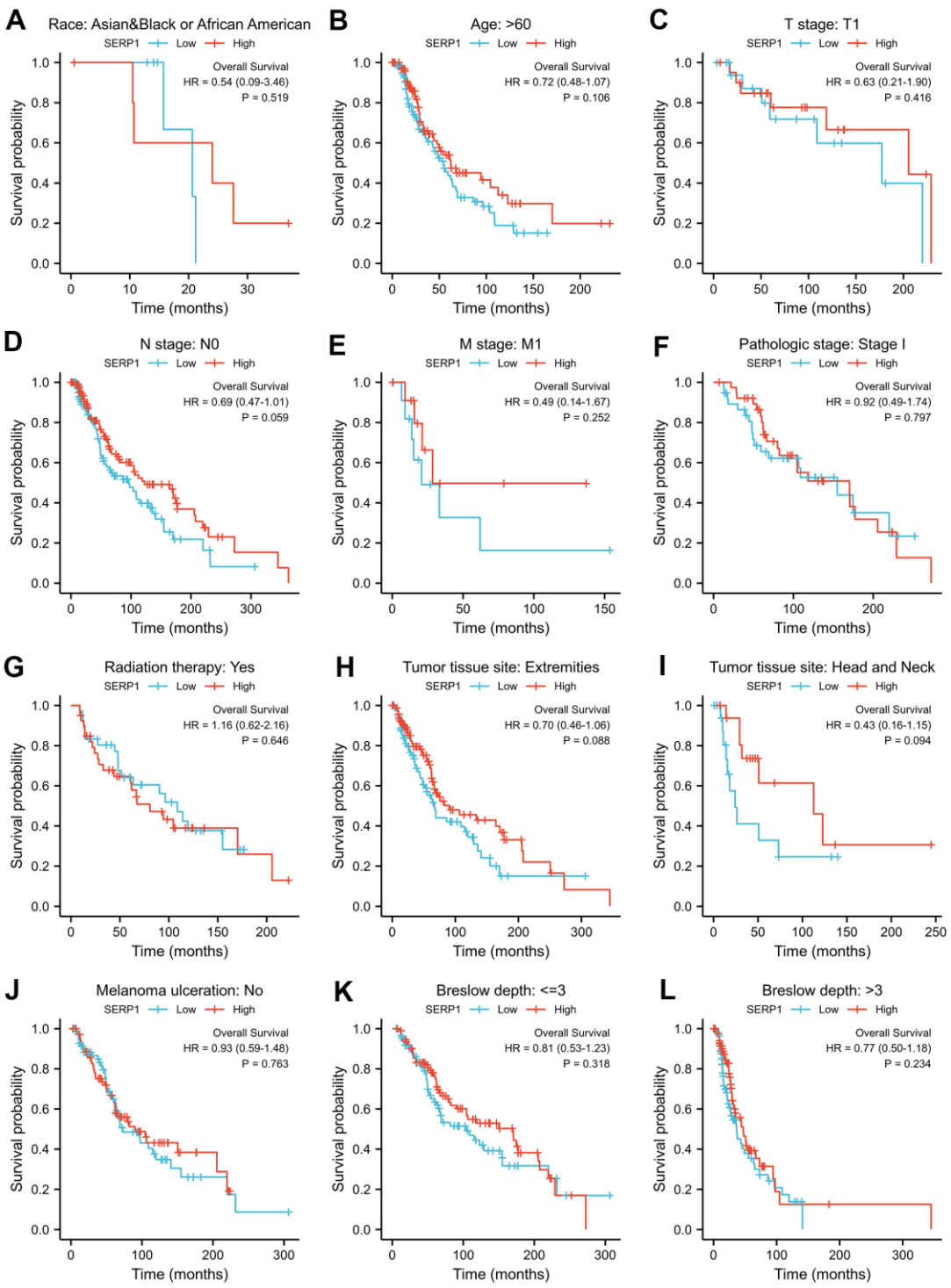
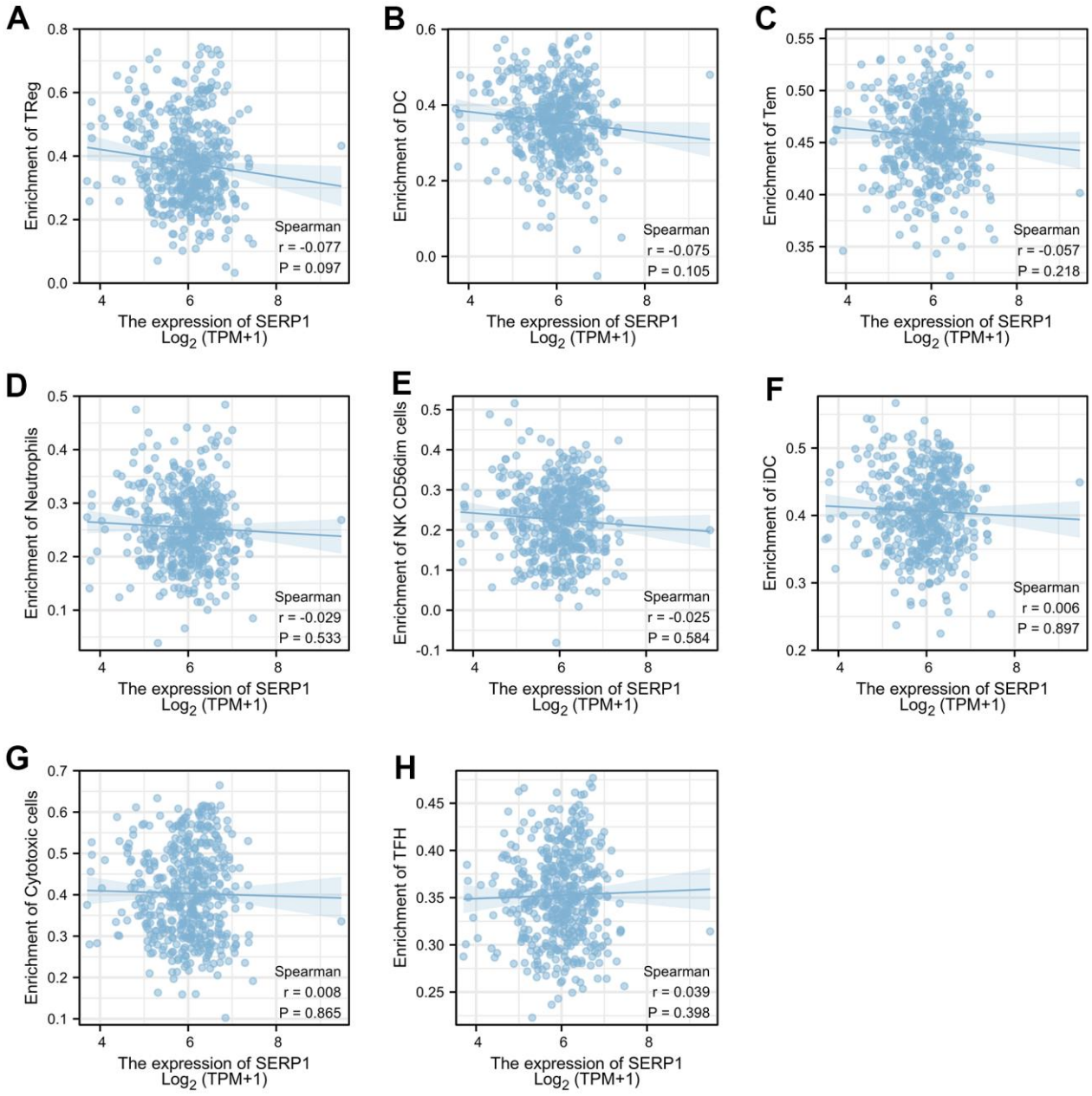
SUPPLEMENTARY MATERIALS

Supplementary Figures



Supplementary Figure 1. The subgroups that high or low SERP1 expression did not show statistical significance on OS. OS Kaplan-Meier curve without statistical significance for (**A**) Asian and Black or African American, (**B**) Age > 60, (**C**) T stage (T1), (**D**) N stage (N0), (**E**) M stage (M1), (**F**) Pathologic Stage (Stage I), (**G**) Radiation therapy Yes, (**H**) Tumor tissue site Extremities, (**I**) Tumor tissue site Head and neck, (**J**) Melanoma ulceration No, (**K**) Breslow depth ≤ 3, (**L**) Breslow depth>3.



Supplementary Figure 2. Correlation of SERP1 and immune cell infiltration in SKCM. The (**A**) Treg, (**B**) DC, (**C**) Tem, (**D**) Neutrophils, (**E**) NK CD56dim cells, (**F**) iDC, (**G**) Cytotoxic cells, (**H**) TFH showed no significant correlation with SERP1.

Supplementary Tables

Supplementary Table 1. TCGA SKCM patient characteristics.

Characteristic	Levels	Overall
n		471
Gender, n (%)	Female	179 (38%)
	Male	292 (62%)
Age, n (%)	<=60	252 (54.4%)
	>60	211 (45.6%)
Race, n (%)	Asian	12 (2.6%)
	Black or African American	1 (0.2%)
	White	448 (97.2%)
Weight, n (%)	<=70	77 (29.7%)
	>70	182 (70.3%)
Height, n (%)	< 170	118 (46.5%)
	>=170	136 (53.5%)
BMI, n (%)	<=25	84 (33.5%)
	>25	167 (66.5%)
T stage, n (%)	T1	41 (11.3%)
	T2	79 (21.7%)
	T3	91 (25%)
	T4	153 (42%)
N stage, n (%)	N0	235 (56.8%)
	N1	74 (17.9%)
	N2	49 (11.8%)
	N3	56 (13.5%)
M stage, n (%)	M0	418 (94.4%)
	M1	25 (5.6%)
Pathologic stage, n (%)	Stage I	77 (18.7%)
	Stage II	140 (34%)
	Stage III	171 (41.5%)
	Stage IV	24 (5.8%)
Radiation therapy, n (%)	No	383 (82.5%)
	Yes	81 (17.5%)
Tumor tissue site, n (%)	Extremities	197 (47%)
	Trunk	171 (40.8%)
	Head and Neck	38 (9.1%)
	Other Specify	13 (3.1%)
Melanoma ulceration, n (%)	No	147 (46.8%)
	Yes	167 (53.2%)
Melanoma Clark level, n (%)	I	6 (1.9%)
	II	18 (5.6%)
	III	77 (23.9%)
	IV	168 (52.2%)
	V	53 (16.5%)
Breslow depth, n (%)	<=3	185 (51.4%)
	>3	175 (48.6%)
Age, median (IQR)		58 (48, 71)

Abbreviations: SERP1, Stress associated endoplasmic reticulum protein 1; SKCM, Skin Cutaneous Melanoma; IQR, interquartile range. TCGA, The Cancer Genome Atlas; BMI, Body Mass Index.

Supplementary Table 2. Top up and down 15 items of differential expressed genes of SERP1.

Gene_name	Gene_id	Gene_biotype	log2foldchange	lfcse	Stat	pvalue	padj
UP							
PASD1	ENSG00000166049	protein_coding	69.83688	4.640308	0.487069	9.527005	1.62E-21
SMR3B	ENSG00000171201	protein_coding	3.471793	4.547842	0.97397	4.669388	3.02E-06
FOXR2	ENSG00000189299	protein_coding	16.68608	4.371976	0.446959	9.781607	1.35E-22
MAEL	ENSG00000143194	protein_coding	81.48709	4.281782	0.310803	13.77653	3.53E-43
LINC00200	ENSG00000229205	lncRNA	5.463603	4.215688	0.522708	8.065098	7.32E-16
OTOR	ENSG00000125879	protein_coding	19.10208	4.166952	0.649502	6.415615	1.40E-10
CT45A1	ENSG00000268940	protein_coding	10.08509	4.113013	0.609996	6.742684	1.55E-11
AL354685.1	ENSG00000233887	processed_pseudogene	8.446124	4.013919	0.705495	5.68951	1.27E-08
SLITRK1	ENSG00000178235	protein_coding	24.69851	3.989265	0.336024	11.87197	1.66E-32
LINC01203	ENSG00000226985	lncRNA	4.358567	3.974891	0.435579	9.125535	7.14E-20
SAGE1	ENSG00000181433	protein_coding	21.23367	3.946295	0.456596	8.642863	5.48E-18
AC004674.1	ENSG00000235592	unprocessed_pseudogene	1.883181	3.786196	0.706633	5.358079	8.41E-08
FAR2P1	ENSG00000180178	transcribed_unprocessed_pseudogene	45.99805	3.684811	0.393731	9.358707	8.07E-21
BX119904.2	ENSG00000230159	lncRNA	7.019114	3.550922	0.666204	5.33008	9.82E-08
HTN3	ENSG00000205649	protein_coding	134.4259	3.549214	0.505356	7.023189	2.17E-12
DOWN							
KRTDAP	ENSG00000188508	protein_coding	924.0061	-4.52021	0.380391	-11.8831	1.45E-32
CDSN	ENSG00000204539	protein_coding	39.63	-4.52433	0.448062	-10.0976	5.66E-24
KRT71	ENSG00000139648	protein_coding	3.876285	-4.6771	0.585631	-7.98642	1.39E-15
SPRR2E	ENSG00000203785	protein_coding	578.3245	-4.68414	0.456065	-10.2708	9.54E-25
LINC01527	ENSG00000224308	lncRNA	16.24596	-4.71413	0.549555	-8.57809	9.65E-18
SPRR2G	ENSG00000159516	protein_coding	324.7711	-4.73287	0.501736	-9.43299	3.99E-21
KRT1	ENSG00000167768	protein_coding	6338.582	-4.75016	0.415758	-11.4253	3.13E-30
KRT16	ENSG00000186832	protein_coding	12867.72	-4.76411	0.39794	-11.9719	4.98E-33
LCE2B	ENSG00000159455	protein_coding	38.90983	-4.82879	0.889447	-5.42898	5.67E-08
CALML5	ENSG00000178372	protein_coding	926.9186	-4.8295	0.495511	-9.74649	1.91E-22
KRT6C	ENSG00000170465	protein_coding	5147.085	-4.89758	0.474097	-10.3303	5.14E-25
WFDC12	ENSG00000168703	protein_coding	54.46971	-4.99446	0.514118	-9.71461	2.61E-22
LCE3E	ENSG00000185966	protein_coding	63.57593	-5.16652	0.631301	-8.18393	2.75E-16
LORICRIN	ENSG00000203782	protein_coding	229.1628	-5.23657	0.489006	-10.7086	9.28E-27
CASP14	ENSG00000105141	protein_coding	734.9536	-5.77555	0.459991	-12.5558	3.69E-36

Abbreviations: SERP1, Stress associated endoplasmic reticulum protein 1.

Supplemental Table 3. Top 50 genes most positively and negatively associated with SERP1 in SKCM patients.

Gene_name	Gene_biotype	cor_pearson	p_pearson	cor_spearman
Top 50 Positive Correlation				
SSR3	protein_coding	0.795181717	5.8818E-104	0.751330735
SLC33A1	protein_coding	0.774519189	2.45527E-95	0.725364812
MYNN	protein_coding	0.76345715	4.33617E-91	0.720671351
ZNF639	protein_coding	0.738031334	3.71758E-82	0.719719153
CNBP	protein_coding	0.752144065	5.57158E-87	0.716621838
UBA5	protein_coding	0.759276657	1.52028E-89	0.711793316
IQCB1	protein_coding	0.76088658	3.89736E-90	0.710512655
TMEM39A	protein_coding	0.748843642	7.99883E-86	0.70747931
NMD3	protein_coding	0.754461278	8.36847E-88	0.703166912
MBD4	protein_coding	0.739794515	9.65449E-83	0.701720296
EIF2A	protein_coding	0.767484819	1.31267E-92	0.696685854
ARMC8	protein_coding	0.744213481	3.13408E-84	0.69225746
ZNF148	protein_coding	0.746288972	6.11349E-85	0.68738656
DCUN1D1	protein_coding	0.742596981	1.10715E-83	0.686714244
PRKCI	protein_coding	0.742134798	1.58548E-83	0.683685263
GTF2E1	protein_coding	0.731596843	4.65073E-80	0.682707569
ZBTB11	protein_coding	0.735595434	2.35205E-81	0.682078551
TBL1XR1	protein_coding	0.740030784	8.05204E-83	0.681496161
ATR	protein_coding	0.741935038	1.85123E-83	0.680610459
NAA50	protein_coding	0.74785842	1.75761E-85	0.679028898
OSBPL11	protein_coding	0.731400519	5.37718E-80	0.674909673
SELENOT	protein_coding	0.767062889	1.89983E-92	0.674804817
PDCD10	protein_coding	0.747620414	2.12461E-85	0.673889944
DHX36	protein_coding	0.73836159	2.89033E-82	0.672835187
PIK3CA	protein_coding	0.71719975	1.40316E-75	0.670906328
RASA2	protein_coding	0.721594824	6.45089E-77	0.670582919
SENP5	protein_coding	0.721900751	5.19471E-77	0.665078521
SEC62	protein_coding	0.725632766	3.61315E-78	0.663001854
TBC1D23	protein_coding	0.722168396	4.29704E-77	0.662420267
MSL2	protein_coding	0.714728218	7.72843E-75	0.661514582
U2SURP	protein_coding	0.713729365	1.53222E-74	0.66061039
FNDC3B	protein_coding	0.701933891	3.99251E-71	0.659742375
KPNA4	protein_coding	0.72348524	1.68414E-77	0.659673352
COMMD2	protein_coding	0.754299399	9.56009E-88	0.657706134
MBNL1	protein_coding	0.72543237	4.17383E-78	0.65721275
RSRC1	protein_coding	0.723014513	2.35539E-77	0.657010848
FYTTD1	protein_coding	0.727606686	8.66561E-79	0.656022358
ATG3	protein_coding	0.720119121	1.82626E-76	0.654352824
POGLUT1	protein_coding	0.701011941	7.26358E-71	0.653684873
SKIL	protein_coding	0.679325958	5.00144E-65	0.651315345
SMC4	protein_coding	0.699104494	2.48722E-70	0.650027563
CDV3	protein_coding	0.684043148	2.96851E-66	0.649527059
NSUN3	protein_coding	0.709181273	3.33211E-73	0.649445058
MRPL3	protein_coding	0.700510064	1.00511E-70	0.648330811
XRN1	protein_coding	0.710267707	1.60547E-73	0.647459924
ZNF267	protein_coding	0.673647668	1.39746E-63	0.64645214
COPB2	protein_coding	0.719733437	2.39442E-76	0.643577054
PCNP	protein_coding	0.711978578	5.04933E-74	0.643065295
ZBTB38	protein_coding	0.690246666	6.66278E-68	0.641416778
IFT57	protein_coding	0.678044334	1.06751E-64	0.641270348
Top 50 negative correlation				
MT-CO3	protein_coding	-0.47468734	7.66882E-28	-0.535968771
MT-CO1	protein_coding	-0.467275484	6.38517E-27	-0.535862422

MT-CO2	protein_coding	-0.463413149	1.88791E-26	-0.525946284
AGPAT2	protein_coding	-0.489282133	1.01214E-29	-0.524308333
MT-ND1	protein_coding	-0.470888194	2.28736E-27	-0.496580654
MTCO1P12	unprocessed_pseudogene	-0.433029919	5.98974E-23	-0.494554749
MT-ATP6	protein_coding	-0.422009854	9.17585E-22	-0.474765195
MT-ND4	protein_coding	-0.437793391	1.78432E-23	-0.471510654
MT-ND3	protein_coding	-0.458969544	6.46072E-26	-0.470042562
MTATP6P1	unprocessed_pseudogene	-0.381584354	8.97534E-18	-0.469923351
MT-CYB	protein_coding	-0.418297813	2.24993E-21	-0.450955069
MT-ND6	protein_coding	-0.423654704	6.14458E-22	-0.443322668
GMPR	protein_coding	-0.42717949	2.5826E-22	-0.441895002
MT-ND5	protein_coding	-0.418753867	2.01638E-21	-0.437519668
C4orf48	protein_coding	-0.429169982	1.57586E-22	-0.430598515
MT-RNR1	Mt_rRNA	-0.399690235	1.7108E-19	-0.429377459
MT-ATP8	protein_coding	-0.406610286	3.5269E-20	-0.422274274
MT-RNR2	Mt_rRNA	-0.387640112	2.4526E-18	-0.41966862
TSPAN10	protein_coding	-0.417326302	2.83999E-21	-0.419311215
MT-TC	Mt_tRNA	-0.442649201	5.08996E-24	-0.413312975
MT-ND4L	protein_coding	-0.409607367	1.75939E-20	-0.410878558
MT-ND2	protein_coding	-0.390802535	1.2323E-18	-0.407848314
MT-TP	Mt_tRNA	-0.39060193	1.28758E-18	-0.402278799
MT-TY	Mt_tRNA	-0.415790896	4.09737E-21	-0.398681022
LGI3	protein_coding	-0.373791557	4.58156E-17	-0.395956283
DIPK1C	protein_coding	-0.364197867	3.21142E-16	-0.39465919
MTCO2P12	unprocessed_pseudogene	-0.330365213	1.86245E-13	-0.39454269
FNDC10	protein_coding	-0.414425212	5.66775E-21	-0.38883685
DPP7	protein_coding	-0.395165978	4.70928E-19	-0.388574195
OSGIN1	protein_coding	-0.365045958	2.71065E-16	-0.385346414
OCA2	protein_coding	-0.420111978	1.45346E-21	-0.382762991
NMRK2	protein_coding	-0.294950356	6.57745E-11	-0.37658248
NT5M	protein_coding	-0.3787931	1.61743E-17	-0.375905513
BAIAP2	protein_coding	-0.339681905	3.48552E-14	-0.371760906
REEP6	protein_coding	-0.378664848	1.66157E-17	-0.37066825
SFTPC	protein_coding	-0.274015405	1.4743E-09	-0.369830026
G6PC3	protein_coding	-0.380325905	1.17131E-17	-0.366047028
ANKRD9	protein_coding	-0.369829757	1.03199E-16	-0.362895964
TPRN	protein_coding	-0.346032998	1.07616E-14	-0.362778476
H2AJ	protein_coding	-0.333179073	1.12944E-13	-0.361549725
MTND1P23	unprocessed_pseudogene	-0.303104672	1.8251E-11	-0.361046913
DIPK1B	protein_coding	-0.369397594	1.12681E-16	-0.359836664
MRPL41	protein_coding	-0.362320507	4.66548E-16	-0.358892276
MFSD12	protein_coding	-0.350747585	4.41996E-15	-0.35865994
PMEL	protein_coding	-0.350992037	4.21893E-15	-0.35765652
MTCO1P40	processed_pseudogene	-0.269445075	2.80958E-09	-0.356970996
MTCO2P22	processed_pseudogene	-0.313498077	3.35737E-12	-0.356818702
SNTA1	protein_coding	-0.345571372	1.17319E-14	-0.352393301
SCARB1	protein_coding	-0.316987843	1.87316E-12	-0.348884948
ABCD1	protein_coding	-0.363286024	3.85134E-16	-0.347318892

Abbreviations: SERP1, Stress associated endoplasmic reticulum protein 1; SKCM, Skin Cutaneous Melanoma.

Supplementary Table 4. SERP1-interaction proteins, annotation of SERP1-interacting proteins and their co-expression scores.

Gene symbol	Annotation	Co-expression scores
SEC61B	Protein transport protein Sec61 subunit beta; Necessary for protein translocation in the endoplasmic reticulum; Belongs to the SEC61-beta family	0.983
SEC61A1	Protein transport protein Sec61 subunit alpha isoform 1; Plays a crucial role in the insertion of secretory and membrane polypeptides into the ER. Required for assembly of membrane and secretory proteins. Tightly associated with membrane- bound ribosomes, either directly or through adapter proteins. Plays a role in pronephric kidney tubule development (By similarity); Belongs to the SecY/SEC61-alpha family	0.974
SEC61G	Protein transport protein Sec61 subunit gamma; Necessary for protein translocation in the endoplasmic reticulum; Belongs to the SecE/SEC61-gamma family	0.959
ASNA1	ATPase ASNA1; ATPase required for the post-translational delivery of tail-anchored (TA) proteins to the endoplasmic reticulum. Recognizes and selectively binds the transmembrane domain of TA proteins in the cytosol. This complex then targets to the endoplasmic reticulum by membrane-bound receptors, where the tail- anchored protein is released for insertion. This process is regulated by ATP binding and hydrolysis. ATP binding drives the homodimer towards the closed dimer state, facilitating recognition of newly synthesized TA membrane proteins. ATP hydrolysis is required for insertion.	0.955
SEC62	Translocation protein SEC62; Required for preprotein translocation	0.954
SEC61A2	Protein transport protein Sec61 subunit alpha isoform 2; Appears to play a crucial role in the insertion of secretory and membrane polypeptides into the ER. It is required for assembly of membrane and secretory proteins. Found to be tightly associated with membrane-bound ribosomes, either directly or through adaptor proteins (By similarity); Belongs to the SecY/SEC61-alpha family	0.945
SEC63	Translocation protein SEC63 homolog; Required for integral membrane and secreted preprotein translocation across the endoplasmic reticulum membrane; DNAJ heat shock proteins	0.945
SPCS1	Signal peptidase complex subunit 1; Component of the microsomal signal peptidase complex which removes signal peptides from nascent proteins as they are translocated into the lumen of the endoplasmic reticulum; Belongs to the SPCS1 family	0.676
SEC11C	Signal peptidase complex catalytic subunit SEC11C; Component of the microsomal signal peptidase complex which removes signal peptides from nascent proteins as they are translocated into the lumen of the endoplasmic reticulum	0.672
DAD1	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit DAD1; Essential subunit of the N-oligosaccharyl transferase (OST) complex which catalyzes the transfer of a high mannose oligosaccharide from a lipid-linked oligosaccharide donor to an asparagine residue within an Asn-X-Ser/Thr consensus motif in nascent polypeptide chains. Required for the assembly of both SST3A- and SS3B-containing OST complexes. Required for efficient N-glycosylation. Loss of the DAD1 protein triggers apoptosis; Belongs to the DAD/OST2 family	0.642

Abbreviations: SERP1, Stress associated endoplasmic reticulum protein 1; SKCM, Skin Cutaneous Melanoma.

Supplementary Table 5. GO analysis and KEGG approach pathway analysis of DEGs between high and low SERP1 in SKCM patients.

Ontology	ID	Description	pvalue	p.adjust	qvalue	Count	GeneID
BP	GO:0030216	keratinocyte differentiation	2.51E-54	1.63E-51	1.49E-51	43	CDSN/DSC1/SFN/IVL/KRT1/KRT2/KRT5/KRT6A/KRT6B/KRT14/KRT16/KRT17/PI3/SERPINB13/PKP1/S100A7/SPRR1A/SPRR1B/SPRR2A/SPRR2B/SPRR2E/SPRR2G/FOXN1/KRT75/CASP14/KLK5/LCE2B/CNFN/LCE3D/SPRR4/KRT6C/LCE1A/LCE1B/LCE1C/LCE1D/LCE1F/LCE2C/LCE2D/LCE3A/LCE3C/LCE3E/LCE6A/C1orf68
BP	GO:0008544	epidermis development	7.02E-53	2.28E-50	2.08E-50	47	CDSN/COL17A1/DSC1/SFN/IVL/KRT1/KRT2/KRT5/KRT6A/KRT6B/KRT14/KRT16/KRT17/PI3/SERPINB13/PKP1/S100A7/SPRR1A/SPRR1B/SPRR2A/SPRR2B/SPRR2E/SPRR2G/FOXN1/KRT75/CASP14/KLK5/LCE2B/CALML5/CNFN/LCE3D/SPRR4/KRT6C/LCE1A/LCE1B/LCE1C/LCE1D/LCE1F/LCE2C/LCE2D/LCE3A/LCE3C/LCE3E/KRTDAP/FLG2/LCE6A/C1orf68
BP	GO:0031424	keratinization	1.09E-52	2.37E-50	2.16E-50	39	CDSN/DSC1/SFN/IVL/KRT1/KRT2/KRT5/KRT6A/KRT6B/KRT14/KRT16/KRT17/PI3/PKP1/SPRR1A/SPRR1B/SPRR2A/SPRR2B/SPRR2E/SPRR2G/KRT75/CASP14/KLK5/LCE2B/CNFN/LCE3D/SPRR4/KRT6C/LCE1A/LCE1B/LCE1C/LCE1D/LCE1F/LCE2C/LCE2D/LCE3A/LCE3C/LCE3E/LCE6A
CC	GO:0001533	cornified envelope	5.83E-53	4.20E-51	3.81E-51	29	CDSN/DSC1/IVL/KRT1/KRT2/PI3/PKP1/SPRR1A/SPRR1B/SPRR2A/SPRR2B/SPRR2E/SPRR2G/LCE2B/CNFN/LCE3D/SPRR4/LCE1A/LCE1B/LCE1C/LCE1D/LCE1F/LCE2C/LCE2D/LCE3A/LCE3C/LCE3E/FLG2/C1orf68
CC	GO:0005882	intermediate filament	1.70E-10	6.13E-09	5.56E-09	12	KRT1/KRT2/KRT5/KRT6A/KRT6B/KRT14/KRT16/KRT17/PKP1/KRT75/CASP14/KRT6C
CC	GO:0045095	keratin filament	3.58E-10	8.59E-09	7.79E-09	9	KRT1/KRT2/KRT5/KRT6A/KRT6B/KRT14/KRT75/CASP14/KRT6C
MF	GO:0030280	structural constituent of epidermis	2.98E-13	3.67E-11	3.29E-11	7	KRT1/KRT2/PI3/PKP1/SPRR1A/SPRR2E/FLG2
MF	GO:0005200	structural constituent of cytoskeleton	3.54E-07	2.18E-05	1.96E-05	7	KRT2/KRT5/KRT6A/KRT6B/KRT14/KRT16/KRT17
MF	GO:0004867	serine-type endopeptidase inhibitor activity	6.28E-05	0.002573	0.002312	5	PI3/SERPINB13/WFDC12/A2ML1/WFDC5
KEGG	hsa04915	Estrogen signaling pathway	5.09E-06	0.000356	0.000123	5	CALML3/KRT14/KRT16/KRT17/CALML5
KEGG	hsa04912	GnRH signaling pathway	2.70E-05	0.000838	0.00029	4	CALML3/CALML5/PLA2G4E/PLA2G4F
KEGG	hsa04750	Inflammatory mediator regulation of TRP channels	3.59E-05	0.000838	0.00029	4	CALML3/CALML5/PLA2G4E/PLA2G4F

Abbreviations: SERP1, Stress associated endoplasmic reticulum protein 1; SKCM, Skin Cutaneous Melanoma; DEGs, differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; MF, molecular function; CC, cellular component.

Supplementary Table 6. Top 50 enrichment plots of GSEA pathway of DEGs between high and low SERP1 in SKCM patients.

ID	pvalue	p.adjust	qvalues
REACTOME_GPCR_LIGAND_BINDING	0.001029	0.039956	0.03215
REACTOME_G_ALPHA_I_SIGNALLING_EVENTS	0.001045	0.039956	0.03215
REACTOME_CLASS_A_1_RHODOPSIN_LIKE_RECEPTORS_	0.00105	0.039956	0.03215
REACTOME_LEISHMANIA_INFECTION	0.001062	0.039956	0.03215
NABA_CORE_MATRISOME	0.001078	0.039956	0.03215
KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	0.001079	0.039956	0.03215
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	0.00108	0.039956	0.03215
WP_GPCRS_CLASS_A_RHODOPSINLIKE	0.001088	0.039956	0.03215
REACTOME_ANTI_INFLAMMATORY_RESPONSE_FAVOURING_LEISHMANIA_PARASITE_INFECTION	0.001094	0.039956	0.03215
REACTOME_G_ALPHA_Q_SIGNALLING_EVENTS	0.001104	0.039956	0.03215
KEGG_CHEMOKINE_SIGNALING_PATHWAY	0.001112	0.039956	0.03215
REACTOME_PEPTIDE_LIGAND_BINDING_RECEPTORS	0.001112	0.039956	0.03215
REACTOME_CELL_SURFACE_INTERACTIONS_AT_THE_VASCULAR_WALL	0.001115	0.039956	0.03215
REACTOME_FC_EPSILON_RECEPTOR_FCERI_SIGNALING	0.001119	0.039956	0.03215
REACTOME_IMMUNOREGULATORY_INTERACTIONS_BETWEEN_A_LYMPHOID_AND_A_NON_LYMPHOID_CELL	0.001119	0.039956	0.03215
REACTOME_FCGAMMA_RECEPTOR_FCGR_DEPENDENT_PHAGOCYTOSIS	0.001153	0.039956	0.03215
REACTOME_SIGNALING_BY_THE_B_CELL_RECEPTOR_BCR_	0.001153	0.039956	0.03215
WP_CHEMOKINE_SIGNALING_PATHWAY	0.001156	0.039956	0.03215
KEGG_JAK_STAT_SIGNALING_PATHWAY	0.001157	0.039956	0.03215
REACTOME_FCERI_MEDIATED_NF_KB_ACTIVATION	0.001164	0.039956	0.03215
KEGG_CELL_ADHESION_MOLECULES_CAMS	0.001167	0.039956	0.03215
REACTOME_COMPLEMENT_CASCADE	0.001192	0.039956	0.03215
REACTOME_PARASITE_INFECTION	0.001198	0.039956	0.03215
REACTOME_BINDING_AND_UPTAKE_OF_LIGANDS_BY_SCAVENGER_RECEPTORS	0.001229	0.039956	0.03215
WP_HUMAN_COMPLEMENT_SYSTEM	0.00123	0.039956	0.03215
REACTOME_FCERI_MEDIATED_MAPK_ACTIVATION	0.001232	0.039956	0.03215
REACTOME_FCGR3A_MEDIATED_IL10_SYNTHESIS	0.001236	0.039956	0.03215
REACTOME_ANTIGEN_ACTIVATES_B_CELL_RECEPTOR_BCR_LEADING_TO_GENERATION_OF_SECOND_MESSENGERS	0.001239	0.039956	0.03215
REACTOME_FCERI_MEDIATED_CA_2_MOBILIZATION	0.001239	0.039956	0.03215
REACTOME_INITIAL_TRIGGERING_OF_COMPLEMENT	0.001241	0.039956	0.03215
WP_ALLOGRAFT_REJECTION	0.001241	0.039956	0.03215
REACTOME_ROLE_OF_PHOSPHOLIPIDS_IN_PHAGOCYTOSIS	0.001247	0.039956	0.03215
WP_TCELL_ANTIGEN_RECEPTOR_TCR_SIGNALING_PATHWAY	0.001248	0.039956	0.03215
KEGG_HEMATOPOIETIC_CELL_LINEAGE	0.001255	0.039956	0.03215
WP_PEPTIDE_GPCRS	0.001255	0.039956	0.03215
KEGG_LEISHMANIA_INFECTION	0.001272	0.039956	0.03215
REACTOME_CREATION_OF_C4_AND_C2_ACTIVATORS	0.001277	0.039956	0.03215
REACTOME_ROLE_OF_LAT2_NTAL_LAB_ON_CALCIUM_MOBILIZATION	0.001277	0.039956	0.03215
REACTOME_FCGR_ACTIVATION	0.001284	0.039956	0.03215
REACTOME_SCAVENGING_OF_HEME_FROM_PLASMA	0.001284	0.039956	0.03215
PID_IL12_2PATHWAY	0.001321	0.039956	0.03215
WP_TCELL_ANTIGEN_RECEPTOR_TCR_PATHWAY_DURING_STAPHYLOCOCCUS_AUREUS_INFECTION	0.001321	0.039956	0.03215
REACTOME_CD22_MEDIATED_BCR_REGULATION	0.001325	0.039956	0.03215
PID_TCR_PATHWAY	0.001328	0.039956	0.03215
PID_CD8_TCR_PATHWAY	0.001346	0.039956	0.03215
REACTOME_INTERLEUKIN_10_SIGNALING	0.001346	0.039956	0.03215
KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	0.001353	0.039956	0.03215
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	0.001353	0.039956	0.03215
REACTOME_INTERLEUKIN_2_FAMILY_SIGNALING	0.001366	0.039956	0.03215
PID_IL12_STAT4_PATHWAY	0.001403	0.039956	0.03215

Abbreviations: SERP1, Stress associated endoplasmic reticulum protein 1; SKCM, Skin Cutaneous Melanoma; DEGs, differentially expressed genes; GSEA, Gene set enrichment analyses.

Supplementary Table 7. Univariate and multivariate Cox regression analysis for clinical variables associated with OS in SKCM patients.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
SERP1 (High vs. Low)	456	0.625 (0.476-0.820)	**<0.001**	0.591 (0.405-0.861)	**0.006**
T stage (T3&T4&T2 vs. T1)	361	2.255 (1.303-3.903)	**0.004**		
N stage (N1&N2&N3 vs. N0)	402	1.752 (1.304-2.354)	**<0.001**	2.945 (1.887-4.597)	**<0.001**
M stage (M1 vs. M0)	430	1.897 (1.029-3.496)	**0.040**		
Gender (Male vs. Female)	456	1.172 (0.879-1.563)	0.281		
Age (>60 vs. <=60)	456	1.656 (1.251-2.192)	**<0.001**	1.204 (0.828-1.750)	0.331
Race (White vs. Asian&Black or African American)	446	0.226 (0.104-0.489)	**<0.001**	0.445 (0.170-1.160)	0.098
Radiation therapy (Yes vs. No)	450	0.977 (0.694-1.377)	0.895		
Melanoma ulceration (Yes vs. No)	313	2.085 (1.495-2.907)	**<0.001**	1.645 (1.062-2.546)	**0.026**
Breslow depth (>3 vs. <=3)	355	2.651 (1.938-3.627)	**<0.001**	2.028 (1.312-3.136)	**0.001**
Pathologic stage (Stage II&Stage III&Stage IV vs. Stage I)	410	1.846 (1.292-2.638)	**<0.001**	0.563 (0.303-1.046)	0.069
BMI (>25 vs. <=25)	241	0.827 (0.513-1.333)	0.436		
Tumor tissue site (Extremities&Head and Neck&Other Specify vs. Trunk)	405	1.147 (0.859-1.532)	0.353		
Melanoma Clark level (III&IV&V vs. I&II)	315	2.689 (1.188-6.088)	**0.018**		

Abbreviations: SERP1, Stress associated endoplasmic reticulum protein 1; SKCM, Skin Cutaneous Melanoma; OS, overall survival; CI, confidence interval. Bold values indicate P < 0.05.

Supplementary Table 8. Univariate and multivariate Cox regression analysis for clinical variables associated with DSS in SKCM patients.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
T stage (T3&T4&T2 vs. T1)	356	2.026 (1.166-3.518)	**0.012**		
N stage (N1&N2&N3 vs. N0)	396	1.665 (1.214-2.283)	**0.002**	2.893 (1.813-4.616)	**<0.001**
M stage (M1 vs. M0)	424	2.200 (1.190-4.069)	**0.012**		
Gender (Male vs. Female)	450	1.161 (0.855-1.575)	0.340		
Age (>60 vs. <=60)	450	1.699 (1.258-2.294)	**<0.001**	1.184 (0.799-1.756)	0.400
Race (White vs. Asian&Black or African American)	440	0.464 (0.146-1.474)	0.193		
Radiation therapy (Yes vs. No)	444	0.994 (0.689-1.433)	0.973		
Melanoma ulceration (Yes vs. No)	309	1.948 (1.372-2.767)	**<0.001**	1.709 (1.087-2.687)	**0.020**
Breslow depth (>3 vs. <=3)	350	2.274 (1.628-3.177)	**<0.001**	1.719 (1.096-2.695)	**0.018**
SERP1 (High vs. Low)	450	0.655 (0.490-0.874)	**0.004**	0.584 (0.395-0.864)	**0.007**
Pathologic stage (Stage II&Stage III&Stage IV vs. Stage I)	405	1.711 (1.181-2.478)	**0.004**	0.586 (0.309-1.109)	0.101
Tumor tissue site (Extremities&Head and Neck&Other Specify vs. Trunk)	399	1.183 (0.868-1.611)	0.288		
BMI (>25 vs. <=25)	238	0.937 (0.545-1.612)	0.815		
Melanoma Clark level (III&IV&V vs. I&II)	310	2.949 (1.207-7.209)	**0.018**		

Abbreviations: SERP1, Stress associated endoplasmic reticulum protein 1; SKCM, Skin Cutaneous Melanoma; DSS, disease specific survival; CI, confidence interval. Bold values indicate P<0.05.

Supplementary Table 9. Univariate and multivariate Cox regression analysis for clinical variables associated with PFI in SKCM patients.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
T stage (T3&T4 vs. T1&T2)	362	1.664 (1.268-2.183)	**<0.001**	1.489 (0.795-2.789)	0.214
N stage (N1&N2&N3 vs. N0)	403	1.870 (1.467-2.385)	**<0.001**	2.925 (1.975-4.334)	**<0.001**
M stage (M1 vs. M0)	431	2.026 (1.255-3.269)	**0.004**	1.519 (0.686-3.364)	0.302
Pathologic stage (Stage II&Stage III&Stage IV vs. Stage I)	411	1.739 (1.293-2.340)	**<0.001**	0.405 (0.195-0.839)	**0.015**
Radiation therapy (Yes vs. No)	451	1.214 (0.917-1.606)	0.175		
Gender (Male vs. Female)	457	1.037 (0.821-1.309)	0.763		
Race (White vs. Asian&Black or African American)	447	0.965 (0.358-2.605)	0.945		
Age (>60 vs. <=60)	457	1.576 (1.240-2.002)	**<0.001**	1.362 (0.971-1.911)	0.074
Melanoma ulceration (Yes vs. No)	313	1.626 (1.228-2.152)	**<0.001**	1.536 (1.040-2.267)	**0.031**
Melanoma Clark level (III&IV&V vs. I&II)	315	1.864 (1.039-3.346)	**0.037**	1.122 (0.551-2.288)	0.751
BMI (>25 vs. <=25)	241	0.966 (0.660-1.416)	0.861		
Breslow depth (>3 vs. <=3)	355	2.032 (1.547-2.669)	**<0.001**	1.557 (1.003-2.417)	**0.048**
SERP1 (High vs. Low)	457	0.768 (0.612-0.964)	**0.023**	0.769 (0.555-1.067)	0.116

Abbreviations: SERP1, Stress associated endoplasmic reticulum protein 1; SKCM, Skin Cutaneous Melanoma; PFI, progress free interval; CI, confidence interval. Bold values indicate P<0.05.

Supplementary Table 10. Details of the ROC information.

Time	Cut-off	Sensitivity	Specificity	Positive predictive value	Negative predictive value
OS					
1 year	6.255538	0.179	0.628	0.0322	0.917
3 year	5.896933	0.516	0.295	0.231	0.598
5 year	6.190405	0.302	0.523	0.308	0.515
DSS					
1 year	6.500816	0.0459	0.789	0.0117	0.938
3 year	5.903546	0.527	0.299	0.208	0.644
5 year	6.190405	0.308	0.52	0.287	0.545

Abbreviations: overall survival: OS; disease specific survival: DSS