

An Integrative Pan-Cancer Analysis Revealing ETS1 as an Oncogenic Immune Protein in Tumor Microenvironment

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Research

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Abstract

Background

Previous research revealed that ETS Proto-Oncogene 1, Transcription Factor (ETS1) might be useful in cancer immunotherapy. However, the processes underlying its therapeutic potential have yet to be thoroughly investigated. The goal of this work was to look into the association between ETS1 expression and immunity and depict its prognostic landscape in pan-cancer.

Methods

The TCGA provides raw data on 33 different types of cancer. GEO gave GSE67501, GSE78220, and IMvigor210. In addition, we looked at ETS1's genetic changes, expression patterns, and survival studies. The researchers investigated the links between ETS1 and the TME and its linkage to immunological processes/elements and the major histocompatibility complex better to understand the importance of ETS1 in cancer immunotherapy. Meanwhile, three distinct immunotherapeutic cohorts were employed to study the relationship between ETS1 and immunotherapeutic response. Finally, PPI analysis and functional gene enrichment were performed using GSEA.

Results

ETS1 expression was shown to be higher in tumor tissue on average. Elevated ETS1 expression has been connected to a worse clinical outcome in patients with OS. ETS1 has been linked to immune cell infiltration, immunological modulators, and immunotherapeutic markers. Furthermore, increased ETS1 expression has been connected to immune-related pathways. However, there was no statistically significant link found between ETS1 and immunotherapeutic response.

Conclusions

ETS1 might be a biomarker for immune infiltration and poor cancer prognosis. It is possible that treatment targets should be researched further.

1. Introduction

ETS1 (ETS Proto-Oncogene 1, Transcription Factor) is a transcription factor that is mainly expressed in lymphoid cells. It is also an oncogene that is commonly elevated in human malignancies from various tissue origins^[1]. Nowadays, ETS1 is becoming increasingly popular as a possible biomarker and essential mediator in various cancers^[3]. According to several studies, ETS1 can impede cell differentiation in various settings and boost its cancer-promoting function by keeping cells immature and proliferating. As a result, ETS1 may help convert drug prospects into therapeutic anticancer strategies^[6]. The link between ETS1 function and carcinogenesis, on the other hand, is yet uncertain, which might be a hot study topic.

The world is today confronted with a significant public health issue: cancer incidence and mortality remain high. Cancer is a problematic sickness because tumors interact with the immune system^[7-1a8a]. The tumor microenvironment (TME) comprises many different types of cells, the bulk of which are invading immune cells. Many studies in recent years have found that TME plays an essential role in the occurrence, development, metastasis, and treatment resistance of human cancers^[9-1a10a]. Understanding the underlying pathways through which TME interacts with immune cells, on the other hand, remains a challenge. Immunotherapy is gaining popularity in many cancer types as research advances, and various checkpoint blocking drugs are already being used in clinical cancer treatment^[11]. As a result, immunophenotypes of tumor-immune interactions and validation of novel immune-associated tumor therapy targets are critical. However, research on ETS1's role in generalized cancer is sparse.

This work aimed to look at the ETS1 expression landscape in 33 different cancers and the underlying impacts on immunological TME. This researcher concentrated on important immune modulators and dynamic immunological indicators such as tumor mutational burden (TMB) and microsatellite instability (MSI). Furthermore, the link between ETS1 expression and immune checkpoint blockade medication was studied. Considering all of these considerations, ETS1 was shown to be a sign of immunological infiltration and a poor prognosis and potential and promising therapeutic target for cancer.

2. Material And Methods

2.1 Acquisition and Processing of Raw Data

We collected Transcriptome RNA-seq and clinical data of 33 tumors from The Cancer Genome Atlas (TCGA)^[12]. ACC, BLCA, STAD, TGCT, DLBC, ESCA, GBM, PRAD, READ, HNSC, LGG, LIHC, KICH, KIRC, KIRP, LUSC, OV, LAML, LUAD, BRCA, COAD, PAAD, THCA, THYM, SKCM, UCEC, and UCS were included in 33 types.

2.2 Genomic Alterations of ETS1 in Cancers

The online cancer genomics database cBioPortal (<http://www.cbioportal.org/>)^[13] was used to determine the change in ETS1 status in cancer patients. ETS1's genomic alterations included copy number amplification, profound loss, an unknown missense mutation, and mRNA overexpression.

2.3 Examining ETS1 Expression in Cancers

The TCGA provides data on ETS1 expression differences between tumor and matched normal tissue. After extracting the ETS1 data with the limma package, we used log₂ (TPM+1) transformed expression data to illustrate the difference analysis findings in parameter selection.

2.4 The relationship between ETS1 and survival and clinical stage

ETS1's impact on cancer survival was determined using overall survival (OS), disease-specific survival (DSS), disease-free survival (DFS), and progression-free survival (PFS). Using log-rank and univariate Cox proportional hazards models, P-values and hazard ratios (HR) with 95 % confidence intervals (CI) for Kaplan-Meier curves were obtained. For multivariate Cox regression, clinical variables such as age, gender, grade, and stage were considered. To study the association between ETS1 expression and clinical stage, the stage survival plots module was employed.

2.5 ETS1 Expression and the Role of Immune Cell Infiltration and the TME

We looked into and calculated the relationship between ETS1 expression and gene markers of tumor-infiltrating immune cells (TIICs) in malignant tumors and discovered some immune cell infiltration. After assessing the TME with the ESTIMATE, the stromalscore, immunescore, and ESTIMATE scores were computed. Tumor purity was shown to be negatively related to the previously reported ratings. The limma was then utilized to evaluate the variances in TME in several cancer samples according to the immunological, ESTIMATE, and stroma scores. To measure tumor cell purity, corresponding scatterplots were constructed. The higher the ImmuneScore or StromalScore predicted score, the higher the immune or stromal ratio. It refers to the more significant the ratio of the relevant component in TME, the higher the associated score. ESTIMATEScore was the sum of the two, showing the percentage of both components in TME.

TMB is gaining traction as a unique and reliable biomarker for predicting immunotherapy response. TMB is calculated as the total number of mutations per DNA megabase, with recognized changes classified as nucleotide insertions, base substitutions, or deletions^[14]. MSI is a molecular tumor feature characterized by the spontaneous loss or gain of nucleotides from short tandem repeat DNA sequences^[15]. The fmsb package was used to investigate the relationship between TMB and MSI.

2.6 Gene Set Enrichment Analysis

To analyze the biological signaling pathway, gene set enrichment analysis (GSEA) was performed in the high and low-expression groups compared to the median level of ETS1 expression. The top four words from the KEGG and GO analyses were shown. KEGG pathways with significant enrichment findings were found using NES (Net enrichment score), gene ratio, and P-value. Enrichment was evaluated significantly for gene sets with $|NES| > 1$, $NOM\ p < 0.05$, and $FDR\ q < 0.05$ ^[16].

2.7 Network of protein-protein interactions

We constructed an ETS1 protein-protein interaction (PPI) network using the GeneMANIA web tool (<http://www.genemania.org>)^[17]. Bioinformatic methodologies distinguish the network integration technique: site prediction, physiological interaction, co-expression, co-localization, gene enrichment analysis, and genetic interaction.

2.8 Immunotherapeutic Response Analysis

As previously stated, this study contained and analyzed three primary independent immunotherapeutic cohorts (GSE78220, GSE67501, and IMvigor210). Immunotherapeutic therapies produced four outcomes in general: complete response (CR), partial response (PR), progressing disease (PD), and stable illness (SD). In this study, patients who achieved CR or PR were defined as responders compared to non-responders who had symptoms of SD or PD. The Wilcoxon test was then used to compare ETS1 expression levels between responder and non-responder groups.

3. Results

The study's goal is to look at the link between ETS1 and immunology and determine its predictive value as a possible biomarker in human cancers. We will investigate the genetic anomalies, expression patterns, and survival assessments of ETS1 expression in pan-cancer patients, as well as its relationship with tumor immune infiltration. Finally, we investigated the connection between PPI and gene functional enrichment.

3.1 Clinical Landscape of ETS1 Expression

ETS1 was expressed differentially in senior GBM patients, as indicated in Figure.1a, albeit it was weakly expressed in BRCA, COAD, ESCA, KIRP, LAML, LUAD, OV, THCA, and UCEC. The data indicated significant gender variations in the expression of BLCA, KIRC, KIRP, LUSC, and PAAD (Figure.1b). Meanwhile, ETS1 expression has been linked to grade stage in numerous cancers, including HNSC, KIRC, LGG, and STAD (Figure.1c). Furthermore, ETS1 expression was significantly related to tumor stage in various cancers, including ACC, BRCA, KICH, KIRC, MESO, STAD, and THCA (Figure.1d).

ETS1 may be an essential new target or biomarker for cancer diagnosis since it can be a sensitive indicator. We looked at ETS1 expression in tumors and neighboring normal tissues to determine if it was linked to cancer. ETS1 mRNA expression was shown to be significantly higher in cancer samples from BLCA, BRCA, CESC, CHOL, COAD, DLBC, GBM, HNSC, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, TGCT, THCA, and UCEC, showing that ETS1 may function as an oncogene in the progression of a range of malignancies (Figure.2a). Figure 2b reveals that the expression levels of DLBC, KIRC, THYM, and SKCM are considerably more significant. As shown in Figure.2c, ETS1 activity was significantly increased in the tumor categories CHOL, ESCA, GBM, HNSC, KIRC, and KIRP, but decreased in the tumor categories BLCA, BRCA, CESC, COAD, GBM, HNSC, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, PAAD, PRAD, READ, THYM, and UCEC. Figure.2d reveals that DLBC, LAML, KIRC, and THYM have much higher levels of activity.

Figure 1. The clinical correlation of ETS1. (a) Age. (b) Gender. (c) Grade. (d) Stage.

Figure 2. Generation and investigation of ETS1 activity. (a) Different analysis of ETS1. (b) the mean expression of ETS1. (c) Different activity analysis of ETS1. (d) the mean activity of ETS1.

3.2 ETS1's Prognostic Value in Cancer

The forest plots (Figure.3) revealed a positive relationship between ETS1 expression and OS in KIRP and MESO but a negative relationship in BLCA, KIRC, and THYM. There was a clear positive connection between ETS1 and DFS in KIRP and PAAD. ETS1 expression was a risk factor in KIRP and MESO but a protective factor in BLCA, KIRC, READ, and THYM in DSS. The PFS forest plot also confirmed ETS1 expression's protective impact in CHOL, KIRC, and THCA and its role as a risk factor in KIRP. However, the plot allowed the researchers to identify other cancers where ETS1 expression was deemed a risk factor, such as KIRP and MESO. Although it was not directly related to clinical features, ETS1 expression was strongly associated with survival in various cancers (BLCA, KIRC, and THYM).

Figure 3. The forest plots of univariate Cox regression analyses.

3.3 ETS1 Expression and Immune Infiltrating Levels in Cancer

We investigated whether ETS1 expression was related to the degree of immune infiltration in various malignancies by evaluating the coefficient of ETS1 expression and immune infiltration level. The stromal and immunological ratings are summarized in Figure 4. ETS1 expression was shown to be associated with the stromal scores ACC, BRCA, CHOL, COAD, ESCA, HNSC, KICH, LIHC, LUAD, LUSC, MESO, OV, PAAD, PCPG, PRAD, READ, STAD, TGCT, THCA, and UCS, as well as the immune scores BRCA, ACC, CHOL, COAD, ESCA, KICH, LUAD, LUSC, PAAD, PRAD, and STAD. As shown in Figure 5, ETS1 expression was negatively associated with Dendritic cells activated in ACC, Macrophages M0 in THYM, Macrophages M2 in THYM, Mast cells resting in THYM, and NK cells activated in THYM, CHOL, and KICH in terms of immune cell infiltration.

Figure 4. Correlation between ETS1 expression and both the ESTIMATE score. (a) StromalScore. (b) ImmuneScore.

Figure 5. Correlation of ETS1 expression with immune infiltration level in cancers

3.4 Analysis of ETS1 expression and immune modulators

The connection between ETS1 expression and immune modulators also was investigated. Figure 6 presents the investigation of 24 various types of immune inhibitors. ETS1 expression was positively related to CSF1R in KICH, KDR in PCPG, and TIGIT in PAAD but negatively connected with PVRL2 in ACC. Correlation investigations of 45 immune stimulators (Figure.7) found that ETS1 expression was positively associated with IL2RA in CHOL, TMEM173 in ACC, and ICOS in PAAD but negatively associated with TNFRSF25 in READ. Furthermore, as shown in Figure.8, ETS1 expression was positively connected to HIA-DOA in ACC, TAP2 in KICH, and HIA-DMA in LUSC, but a negative connection with HIA-G in READ.

Figure 6. The expression correlation between ETS1 and immune inhibitors. Red indicates positive correlation whereas blue indicates negative correlation.

Figure 7. The expression correlation between ETS1 and immune stimulators. Red indicates positive correlation whereas blue indicates negative correlation.

Figure 8. The expression correlation between ETS1 and MHC molecules. Red indicates positive correlation whereas blue indicates negative correlation.

3.5 Immunotherapeutic markers and response of ETS1

The connection with ETS1 and two novel dynamic markers of immune checkpoint blockage (TMB and MSI) was studied further. Figure.9 shows that ETS1 expression is positively associated with TMB in CESC, BRCA, BLCA, UCEC, KIRC, LAML, LGG, LUAD, LUSC, PCPG, SARC, THCA, TGCT, and STAD but negatively related to CHOL, DLBC, HNSC, KIRP, LIHC, PAAD, and THYM. MSI was shown to have a positive association in BLCA, BRCA, CESC, KIRC, LAML, LGG, LUAD, LUSC, PCPG, SARC, TGCT, THCA, and UCEC and a negative association in CHOL, DLBC, HNSC, KIRP, LIHC, PAAD, STAD, and THYM.

Figure.9 shows no significant difference in ETS1 expression between the responder and non-responder groups in the three different cohorts. In the studied cohorts, patients with lower ETS1 expression were shown to be more susceptible to immunotherapy.

Figure 9. The correlation between ETS1 and both immunotherapeutic markers and response.

3.6 PPI Network of ETS1 in Cancers and GSEA

Following that, we built an ETS1 PPI network to investigate the fundamental mechanisms that ETS1 plays in cancer carcinogenesis (Figure.10). As seen in the figure, ETS1 made firm physical contact with SP100, required for cancer metastasis. SP100 (SP100 Nuclear Antigen) is a gene that codes for proteins. Functions as a transcriptional coactivator of ETS1 and ETS2 are involved in various physiological processes such as cell proliferation, differentiation, and apoptosis^[18]. It may also serve as a corepressor of ETS1, preventing it from binding to DNA under certain situations^[19]. ETS1 regulation may play a function in angiogenesis by modulating endothelial cell motility and invasion^[20]. ETS1 was also expected to be related to SP1 and CAMK2G. The functional enrichment of high ETS1 expression versus low ETS1 expression was then determined using GSEA (Figure.11). According to the KEGG enrichment term, high expression of ETS1 mainly was connected with metabolic-related activities such as cytosolic dna sensing pathway, metabolism of xenobiotics by cytochrome p450, olfactory transduction, retinol metabolism, and steroid hormone biosynthesis. According to the GO enrichment term, high expression of ETS1 is mainly linked with detection of chemical stimulus, detection of stimulus involved in sensory perception, epidermis development, sensory perception of chemical stimulus, and skin development.

Figure 10. PPI network of ETS1.

Figure 11. GSEA for samples with ETS1 expression. (a) The low expression. (b) The high expression sample. (c) The low expression. (d) The high expression. (a +b): The enriched gene sets in KEGG. (c + d): The enriched gene sets in GO.

4. Discussion

Contrary to conventional perception, ETS1 is a toxicant-related transcription factor that plays an essential role in the immunological TME and may have immunotherapeutic potential. Thus, more ETS1-related research involving the TME, immune cells, immunological modulators, and the immunotherapeutic response is necessary. This research aimed to understand more about the pathways that may link ETS1 to immune-related factors in pan-cancer. First, the association between ETS1 and clinical variables was investigated, and no significant differences in age, gender, or tumor stage were discovered in most cancer types, confirming previous findings. ETS1 expression, on the other hand, has only a marginal prognostic value in a variety of cancers, including gastric cancer (GC)^[21]. Similarly, previous research has identified ETS1 as a proto-oncogene in various cancers, including hepatocellular carcinoma^[22], colorectal cancer^[23], and Cervical Cancer Malignancy^[24]. The RHPN1-AS1/miR-1299/ETS1 positive feedback loop accelerates GC degradation^[25]. ETS1 promotes epithelial-to-mesenchymal transition and enhances transforming growth factor signaling in prostate cancer cells^[26]. We predict that therapeutic modification of ETS1 activity in different tumor types may be a realistic clinical strategy based on the evidence given here, which reveals the usage of ETS1 in cancer prognosis.

Furthermore, when the transcriptional level was compared to the ETS1 activity score, the transcription level partially matched the total ETS1 activation in several tumors (BLCA, BRCA, CESC, COAD, GBM, HNSC, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, and UCEC), indicating that transcription level represented ETS1 activation in these tumors. ETS1 expression and activity were inconsistent in several cancers (PAAD, THYM, CHOL, DLBC, TGCT, and THCA). This might be due to ETS1 expression being influenced by post-transcriptional protein modification or protein metabolism.

The link between ETS1 and immune-cell infiltration was examined further to evaluate ETS1's potential use. ETS1 and M2 and M0 macrophages were discovered to have a significant connection in THYM. Moreover, some previous study indicates that ETS1 impacts tumor growth and immune responses inside TME-associated macrophages^[27]. ETS1 may be involved in macrophage polarization and the subsequent activation of an immunosuppressive response^[28]. KDR would have the most significant adverse connection with ETS1 in PCPG. Except for UVM, most immune stimulants and MHC molecules showed a positive relationship with ETS1; this exciting finding may lead to the identification of a novel regulatory mechanism in UVM immunotherapy. Enrichment analysis further indicated that high ETS1 expression was mainly associated with metabolic-related activities. Dysregulation of cytokine and adipocytokine expression in adipose tissue characterizes metabolic inflammation^[29]. ETS1 functions as a transcription factor. Directly regulates the expression of cytokine and chemokine genes in a range of cellular settings^[30]. This protein may influence lymphoid cell development, survival, and proliferation and cause inflammatory molecules to clump together, making it easier for macrophages to enter^[31]. Increased ETS1 expression, according to the current findings, may impact innate immunity in some cancers by activating metabolic-related mechanisms.

Furthermore, in this study, two immunotherapeutic biomarkers (TMB and MSI) were found to have a significant connection with AhR in various cancers. The TMB provides a decent estimate of tumor-

neoantigen burden; in general, the more somatic mutations a tumor has, the more likely it is to create neoantigens^[32]. On the other hand, MSI is described as a robust mutator phenotype caused by poor DNA mismatch repair and is a possible prognostic indicator for immunotherapy.^[33] ETS1 was adversely associated with TMB and MSI in CHOL, DLBC, HNSC, KIRP, LIHC, PAAD, and THYM; however, it was positively associated with both biomarkers in BLCA, BRCA, CESC, KIRC, LAML, LGG, LUAD, LUSC, PCPG, SARC, TGCT, THCA, and UCEC. This showed that ETS1 might have an indirect effect on the immunotherapeutic response of the previous malignancies. The link between ETS1 and the immunotherapeutic response was investigated, but no significant differences were found in any cohorts studied. As a result, our findings shed insight on ETS1's latent involvement in tumor immunology and its potential application as a cancer biomarker. Meanwhile, only three relevant cohorts were studied in our investigation of immunotherapeutic response, making it difficult to define the precise immunotherapeutic response of ETS1. More relevant immunotherapeutic populations should be studied in the future.

This research sheds new information on the role of ETS1 in cancer immunotherapy. It suggests a link between ETS1 and important immunological indicators, which might help us comprehend the possible connections between ETS1 and the immune system. Despite the fact that it is offered for theoretical underpinnings and analysis ideas, our study has limitations. Using the TCGA datasets, we first generate a validated ETS1 prediction signature. We are frequently unable to obtain adequate external information from several publicly available sources. Second, while the bioinformatics analysis provided us with some valuable insights into the role of ETS1 in cancer, these findings are preliminary. Biological research, either in vitro or in vivo, is needed to confirm our findings and improve therapeutic utility. Finally, post-translational modifications are essential in altering intracellular signaling and regulatory factor function^[34][a35a]; unfortunately, post-translational modification information for LCN2 is not available in these databases. However, to thoroughly understand the facts presented above, we shall do an extra investigation.

5. Conclusion

In conclusion, our research highlighted the close link and prognostic significance of ETS1 expression in many human cancers. ETS1 might be a viable new cancer therapeutic target. Furthermore, our findings provide insight on ETS1's important role in carcinogenesis and metastasis, as well as a potential mechanism through which ETS1 expression might influence tumor immunology and metabolic activity. Our findings are expected to aid in the discovery of the link between ETS1 expression and immunological TME, allowing us to learn more about their potential role in cancer genesis and development and, as a result, offer immuno-based anti-cancer therapy.

Abbreviations

ETS1	ETS Proto-Oncogene 1, Transcription Factor	TCGA	The Cancer Genome Atlas
GO	Gene Ontology	GEO	Gene Expression Omnibus
KEGG	Kyoto Encyclopedia of Genes and Genomes	GSEA	Gene set enrichment analysis
TME	tumor microenvironment	MSI	microsatellite instability
TMB	tumor mutational burden	OS	overall survival
DSS	disease-specific survival	CI	confidence intervals
HR	hazard ratios	TIICs	tumor-infiltrating immune cells
PPI	protein-protein interaction	PFs	progression-free survival
CR	complete response	PR	partial response
PD	progressing disease	SD	and stable illness

Declarations

Data availability

Patients who granted informed consent to use their data have been uploaded to the public-accessible TCGA databases. At their leisure, users can get and publish relevant articles depending on the needed data. Our study has no ethical problems or conflicts of interest because it is based on open-source data.

Ethics approval and consent to participation

This manuscript is not a clinical trial, hence the ethics approval and consent to participation is not applicable.

Consent for publication

All authors have read and approved this manuscript to be considered for publication.

Competing interests

The authors declare no competing financial interests.

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Author Contributions

Zixuan Wu drafted and revised the manuscript. Xuyan Huang and Minjie Cai are in charge of data collection. Peidong Huang conceived and designed this article, in charge of syntax modification and revise of the manuscript. Zunhui Guan revised the manuscript. All the authors have read and agreed to the final version manuscript.

References

- 1 [1] Testoni, M., Chung, E.Y., Priebe, V. and Bertoni, F. (2015) The transcription factor ETS1 in lymphomas: friend or foe? *Leuk Lymphoma* 56, 1975-1980, 10.3109/10428194.2014.981670
- 2 [1] Nazir, S.U., Kumar, R., Singh, A., Khan, A., Tanwar, P. and Tripathi, R. et al. (2019) Breast cancer invasion and progression by MMP-9 through Ets-1 transcription factor. *Gene* 711, 143952, 10.1016/j.gene.2019.143952
- 3 [1] Mao, S., Zhang, W., Yang, F., Guo, Y., Wang, H. and Wu, Y. et al. (2021) Hsa_circ_0004296 inhibits metastasis of prostate cancer by interacting with EIF4A3 to prevent nuclear export of ETS1 mRNA. *J Exp Clin Cancer Res* 40, 336, 10.1186/s13046-021-02138-8
- 4 [1] Liu, O., Wang, C., Wang, S., Hu, Y., Gou, R. and Dong, H. et al. (2021) Keratin 80 regulated by miR-206/ETS1 promotes tumor progression via the MEK/ERK pathway in ovarian cancer. *J. Cancer* 12, 6835-6850, 10.7150/jca.64031
- 5 [1] Kori, M., Gov, E. and Arga, K.Y. (2019) Novel Genomic Biomarker Candidates for Cervical Cancer As Identified by Differential Co-Expression Network Analysis. *Omics* 23, 261-273, 10.1089/omi.2019.0025
- 6 [1] Garrett-Sinha, L.A. (2013) Review of Ets1 structure, function, and roles in immunity. *Cell. Mol. Life Sci.* 70, 3375-3390, 10.1007/s00018-012-1243-7

- 7 [1] Abbott, M. and Ustoyev, Y. (2019) Cancer and the Immune System: The History and Background of Immunotherapy. *Semin Oncol Nurs* 35, 150923, 10.1016/j.soncn.2019.08.002
- 8 [1] Prieto, L.I. and Baker, D.J. (2019) Cellular Senescence and the Immune System in Cancer. *Gerontology* 65, 505-512, 10.1159/000500683
- 9 [1] Arneth, B. (2019) Tumor Microenvironment. *Medicina (Kaunas)* 56, 10.3390/medicina56010015
- 10 [1] Kaymak, I., Williams, K.S., Cantor, J.R. and Jones, R.G. (2021) Immunometabolic Interplay in the Tumor Microenvironment. *Cancer Cell* 39, 28-37, 10.1016/j.ccell.2020.09.004
- 11 [1] Tan, S., Li, D. and Zhu, X. (2020) Cancer immunotherapy: Pros, cons and beyond. *Biomed. Pharmacother.* 124, 109821, 10.1016/j.biopha.2020.109821
- 12 [1] Wang Zhining, Jensen Mark A, Zenklusen Jean Claude. A Practical Guide to The Cancer Genome Atlas (TCGA). [J]. *Methods in molecular biology* (Clifton, N.J.), 2016, 1418:
- 13 [1] Cerami, E., Gao, J., Dogrusoz, U., Gross, B.E., Sumer, S.O. and Aksoy, B.A. et al. (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* 2, 401-404, 10.1158/2159-8290.CD-12-0095
- 14 [1] Greillier, L., Tomasini, P. and Barlesi, F. (2018) The clinical utility of tumor mutational burden in non-small cell lung cancer. *Transl Lung Cancer Res* 7, 639-646, 10.21037/tlcr.2018.10.08
- 15 [1] Hause, R.J., Pritchard, C.C., Shendure, J. and Salipante, S.J. (2016) Classification and characterization of microsatellite instability across 18 cancer types. *Nat. Med.* 22, 1342-1350, 10.1038/nm.4191
- 16 [1] Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L. and Gillette, M.A. et al. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 102, 15545-15550, 10.1073/pnas.0506580102
- 17 [1] Franz, M., Rodriguez, H., Lopes, C., Zuberi, K., Montojo, J. and Bader, G.D. et al. (2018) GeneMANIA update 2018. *Nucleic Acids Res.* 46, W60-W64, 10.1093/nar/gky311
- 18 [1] Wasylyk, C., Schlumberger, S.E., Criqui-Filipe, P. and Wasylyk, B. (2002) Sp100 interacts with ETS-1 and stimulates its transcriptional activity. *Mol. Cell. Biol.* 22, 2687-2702, 10.1128/MCB.22.8.2687-2702.2002
- 19 [1] Yordy, J.S., Li, R., Sementchenko, V.I., Pei, H., Muise-Helmericks, R.C. and Watson, D.K. (2004) SP100 expression modulates ETS1 transcriptional activity and inhibits cell invasion. *Oncogene* 23, 6654-6665, 10.1038/sj.onc.1207891

- 20 [1] Yordy, J.S., Moussa, O., Pei, H., Chaussabel, D., Li, R. and Watson, D.K. (2005) SP100 inhibits ETS1 activity in primary endothelial cells. *Oncogene* 24, 916-931, 10.1038/sj.onc.1208245
- 21 [1] Mei, D., Qi, Y., Xia, Y., Ma, J., Hu, H. and Ai, J. et al. (2021) Microarray profile analysis identifies ETS1 as potential biomarker regulated by miR-23b and modulates TCF4 in gastric cancer. *World J. Surg. Oncol.* 19, 311, 10.1186/s12957-021-02417-w
- 22 [1] Chen, Y., Peng, C., Chen, J., Chen, D., Yang, B. and He, B. et al. (2019) WTAP facilitates progression of hepatocellular carcinoma via m6A-HuR-dependent epigenetic silencing of ETS1. *Mol. Cancer* 18, 127, 10.1186/s12943-019-1053-8
- 23 [1] Gu, C., Cai, J., Xu, Z., Zhou, S., Ye, L. and Yan, Q. et al. (2019) MiR-532-3p suppresses colorectal cancer progression by disrupting the ETS1/TGM2 axis-mediated Wnt/beta-catenin signaling. *Cell Death Dis.* 10, 739, 10.1038/s41419-019-1962-x
- 24 [1] Liu, J., Li, D., Zhang, X., Li, Y. and Ou, J. (2020) Histone Demethylase KDM3A Promotes Cervical Cancer Malignancy Through the ETS1/KIF14/Hedgehog Axis. *Onco Targets Ther* 13, 11957-11973, 10.2147/OTT.S276559
- 25 [1] Ding, L., Wang, L., Li, Z., Jiang, X., Xu, Y. and Han, N. (2020) The positive feedback loop of RHPN1-AS1/miR-1299/ETS1 accelerates the deterioration of gastric cancer. *Biomed. Pharmacother.* 124, 109848, 10.1016/j.biopha.2020.109848
- 26 [1] Rodgers, J.J., McClure, R., Epis, M.R., Cohen, R.J., Leedman, P.J. and Harvey, J.M. et al. (2019) ETS1 induces transforming growth factor beta signaling and promotes epithelial-to-mesenchymal transition in prostate cancer cells. *J. Cell. Biochem.* 120, 848-860, 10.1002/jcb.27446
- 27 [1] Mei, D., Qi, Y., Xia, Y., Ma, J., Hu, H. and Ai, J. et al. (2021) Microarray profile analysis identifies ETS1 as potential biomarker regulated by miR-23b and modulates TCF4 in gastric cancer. *World J. Surg. Oncol.* 19, 311, 10.1186/s12957-021-02417-w
- 28 [1] He, L., Liu, L., Li, T., Zhuang, D., Dai, J. and Wang, B. et al. (2021) Exploring the Imbalance of Periodontitis Immune System From the Cellular to Molecular Level. *Front Genet* 12, 653209, 10.3389/fgene.2021.653209
- 29 [1] Wu, H. and Ballantyne, C.M. (2017) Skeletal muscle inflammation and insulin resistance in obesity. *J. Clin. Invest.* 127, 43-54, 10.1172/JCI88880
- 30 [1] Taveirne, S., Wahlen, S., Van Loocke, W., Kiekens, L., Persyn, E. and Van Ammel, E. et al. (2020) The transcription factor ETS1 is an important regulator of human NK cell development and terminal differentiation. *Blood* 136, 288-298, 10.1182/blood.2020005204
- 31 [1] Dittmer, J. (2015) The role of the transcription factor Ets1 in carcinoma. *Semin. Cancer Biol.* 35, 20-38, 10.1016/j.semcancer.2015.09.010

32 [1] Chan, T.A., Yarchoan, M., Jaffee, E., Swanton, C., Quezada, S.A. and Stenzinger, A. et al. (2019) Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. *Ann. Oncol.* 30, 44-56, 10.1093/annonc/mdy495

33 [1] Yamamoto, H. and Imai, K. (2019) An updated review of microsatellite instability in the era of next-generation sequencing and precision medicine. *Semin. Oncol.* 46, 261-270, 10.1053/j.seminoncol.2019.08.003

34 [1] Berg, V., Rusch, M., Vartak, N., Jungst, C., Schauss, A. and Waldmann, H. et al. (2015) miRs-138 and -424 control palmitoylation-dependent CD95-mediated cell death by targeting acyl protein thioesterases 1 and 2 in CLL. *Blood* 125, 2948-2957, 10.1182/blood-2014-07-586511

35 [1] Izquierdo, J.M. (2010) Heterogeneous ribonucleoprotein C displays a repressor activity mediated by T-cell intracellular antigen-1-related/like protein to modulate Fas exon 6 splicing through a mechanism involving Hu antigen R. *Nucleic Acids Res.* 38, 8001-8014, 10.1093/nar/gkq698

Figures

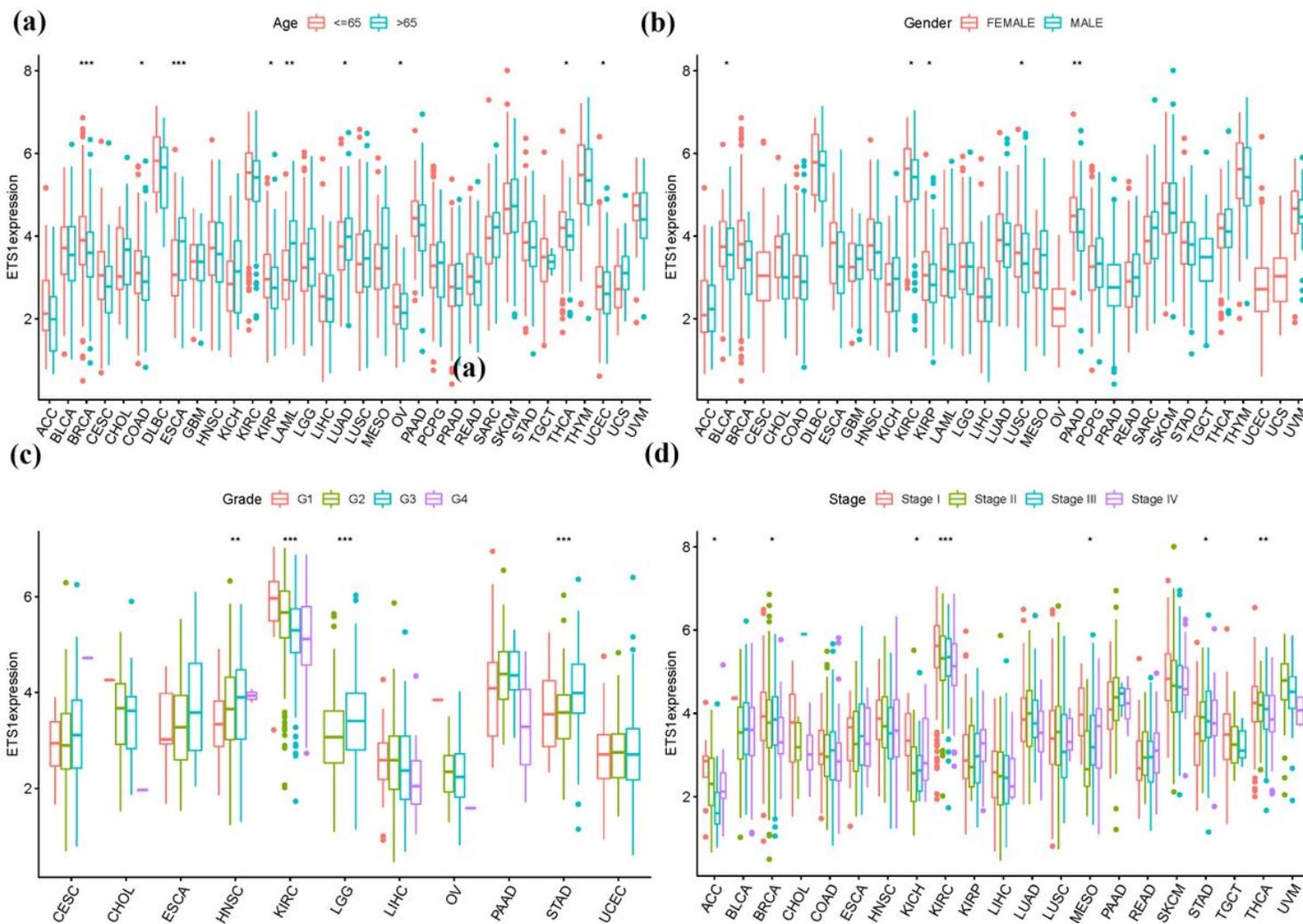


Figure 1

The clinical correlation of ETS1. (a) Age. (b) Gender. (c) Grade. (d) Stage.

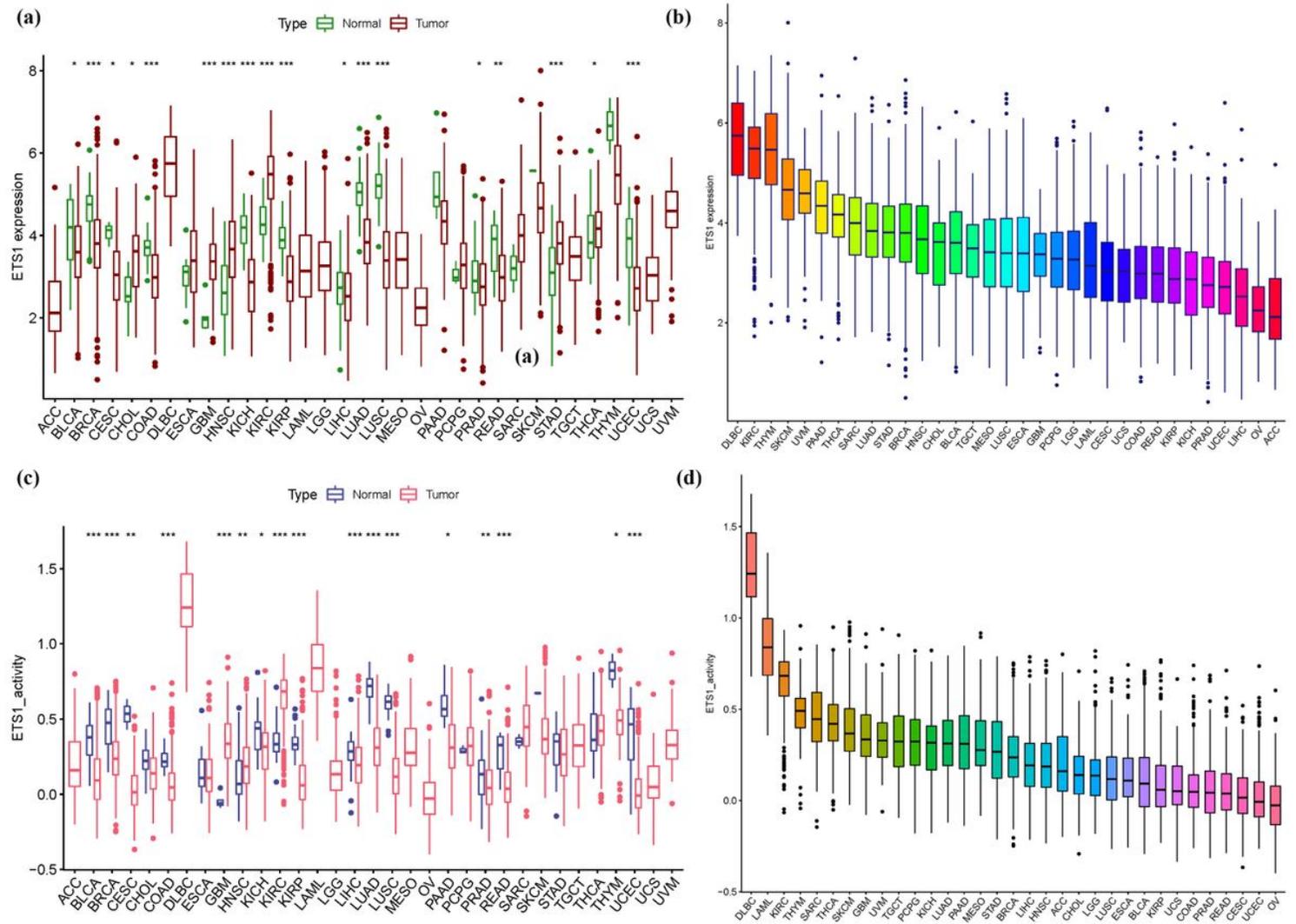


Figure 2

Generation and investigation of ETS1 activity. (a) Different analysis of ETS1. (b) the mean expression of ETS1. (c) Different activity analysis of ETS1. (d) the mean activity of ETS1.

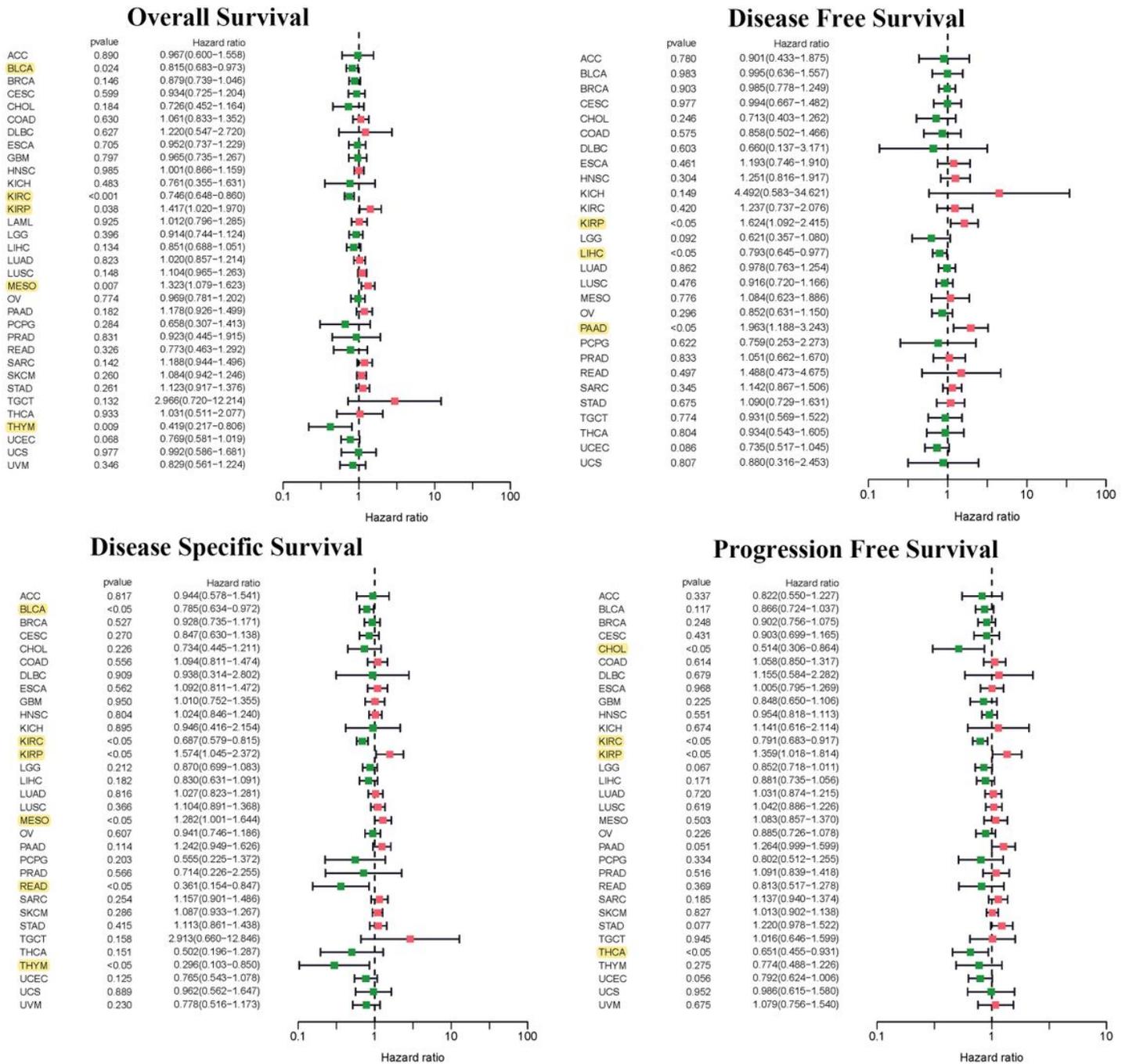


Figure 3

The forest plots of univariate Cox regression analyses.

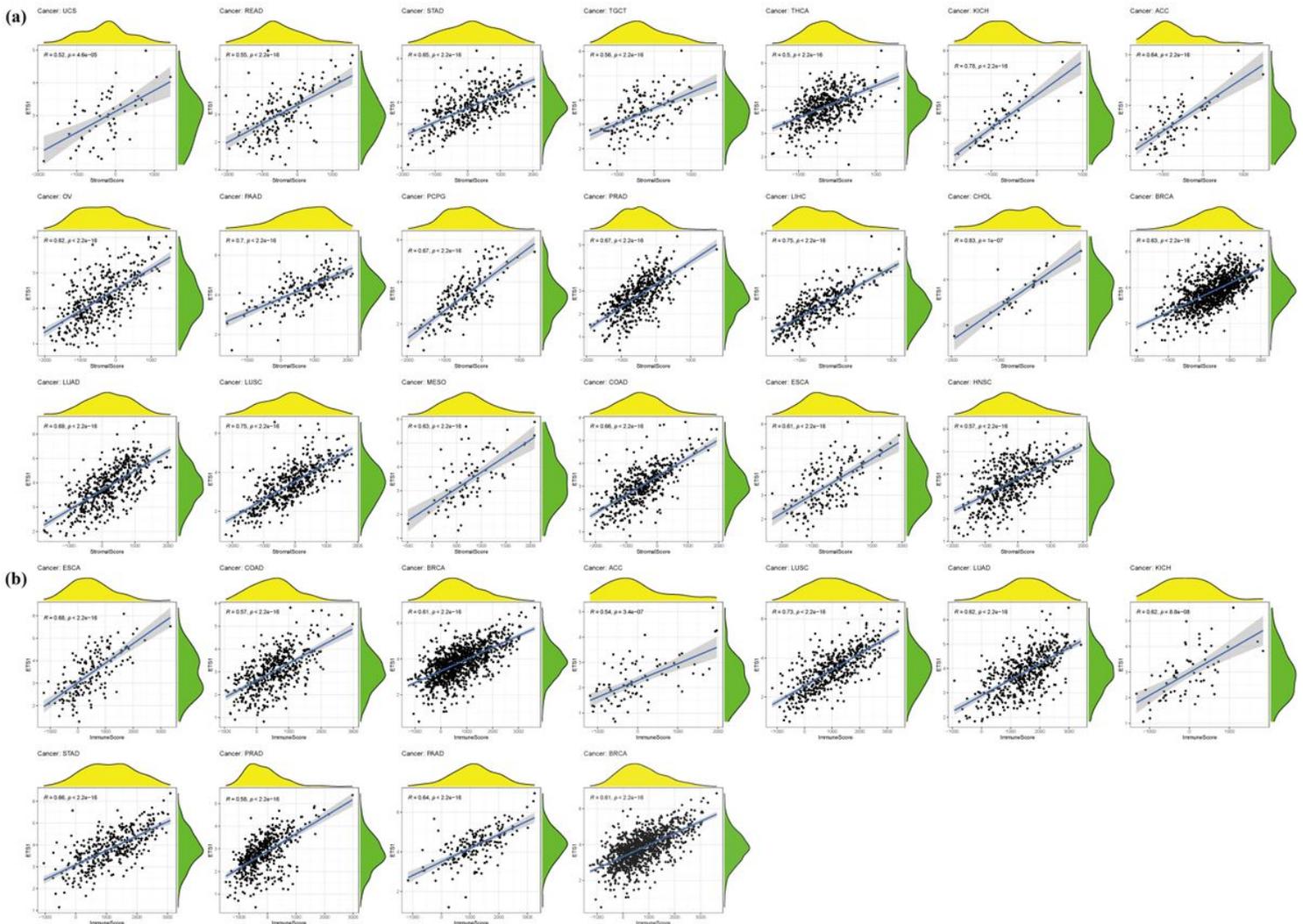


Figure 4

Correlation between ETS1 expression and both the ESTIMATE score. (a) StromaScore. (b) ImmuneScore.

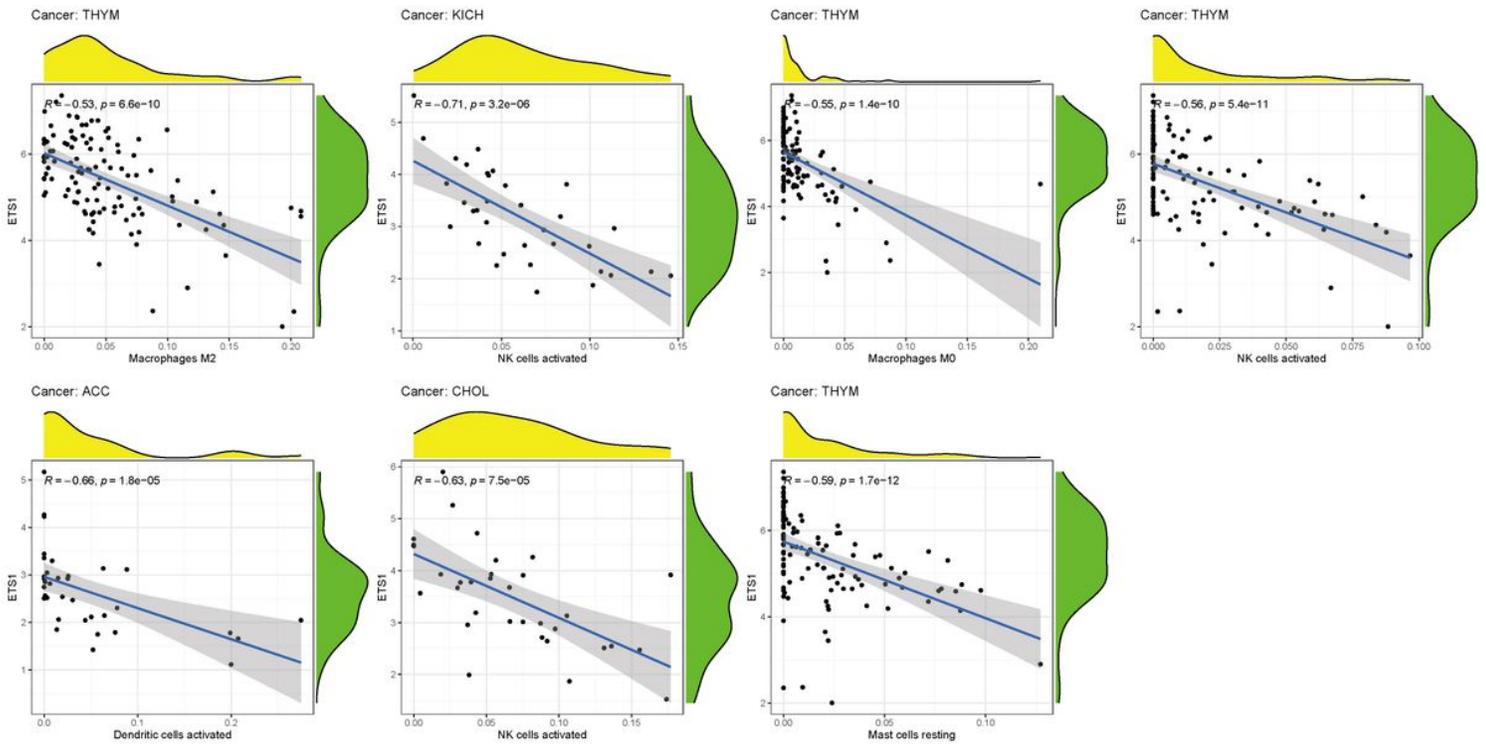


Figure 5

Correlation of ETS1 expression with immune infiltration level in cancers

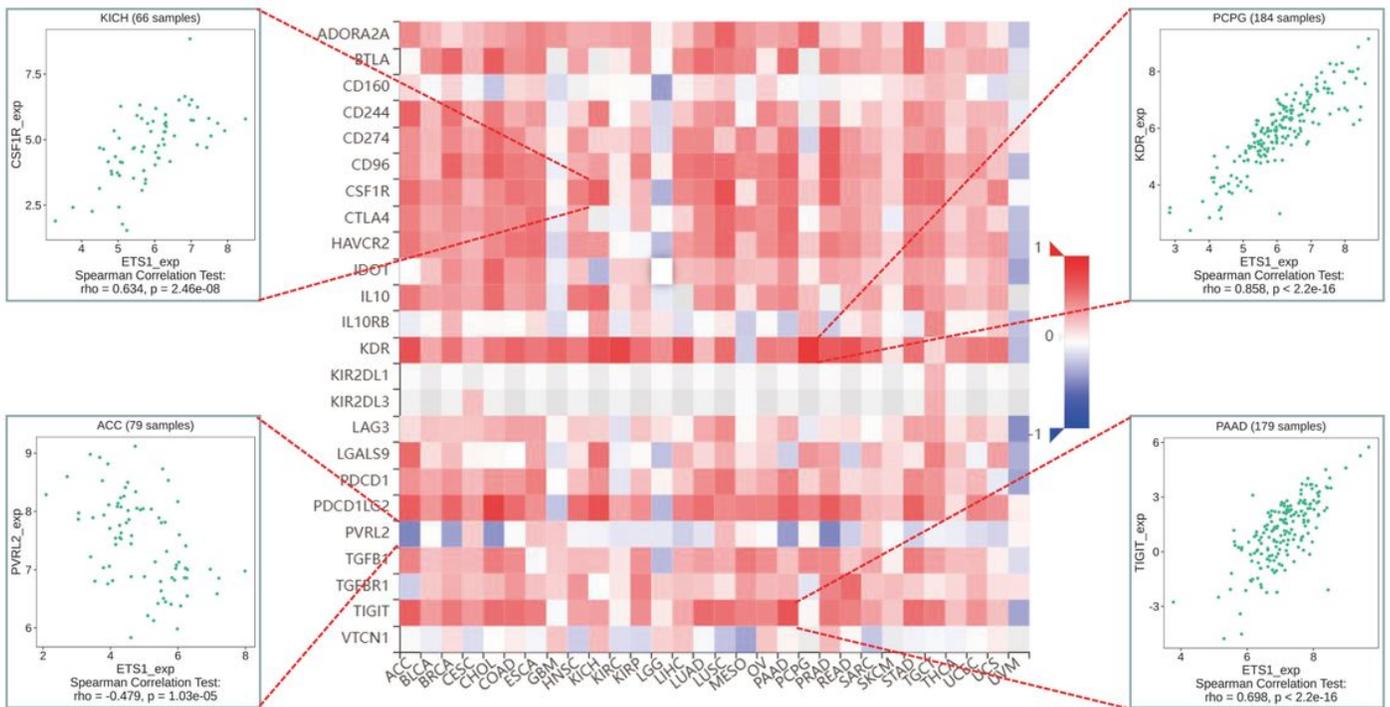


Figure 6

The expression correlation between ETS1 and immune inhibitors. Red indicates positive correlation whereas blue indicates negative correlation.

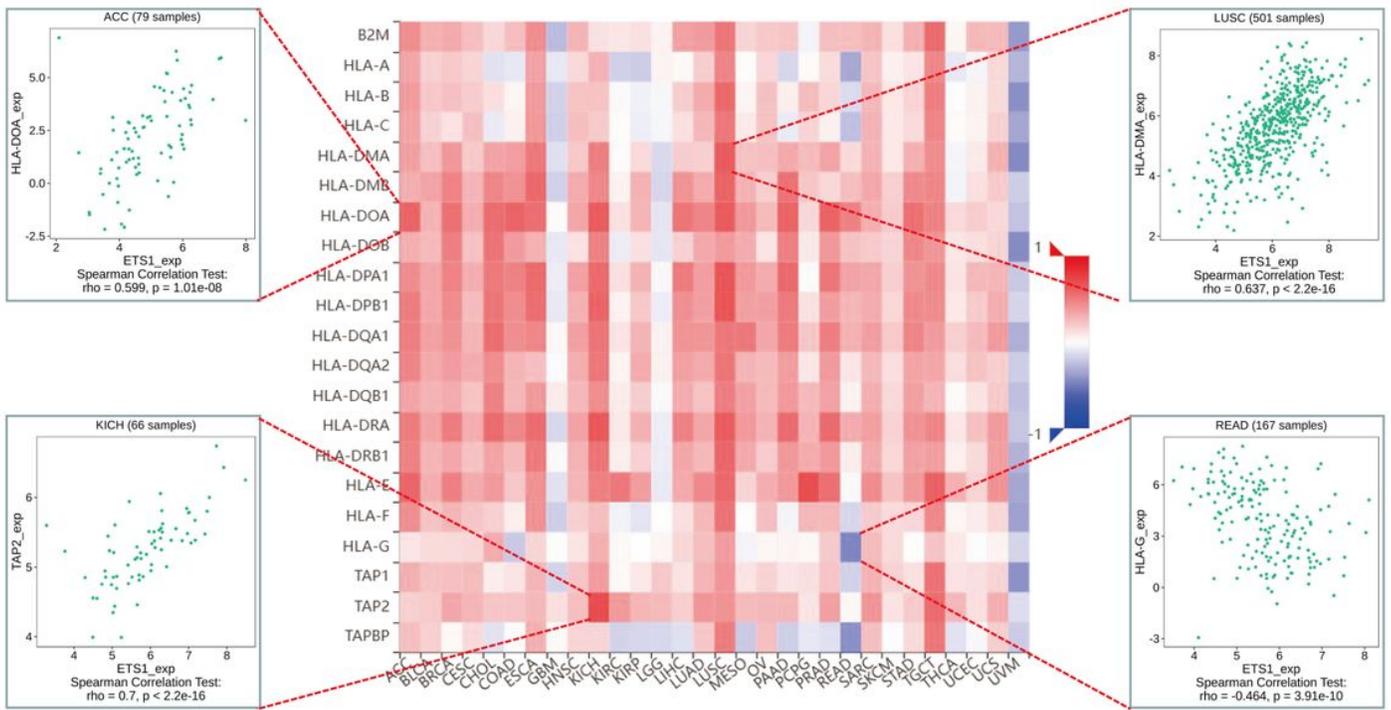


Figure 8

The expression correlation between ETS1 and MHC molecules. Red indicates positive correlation whereas blue indicates negative correlation.

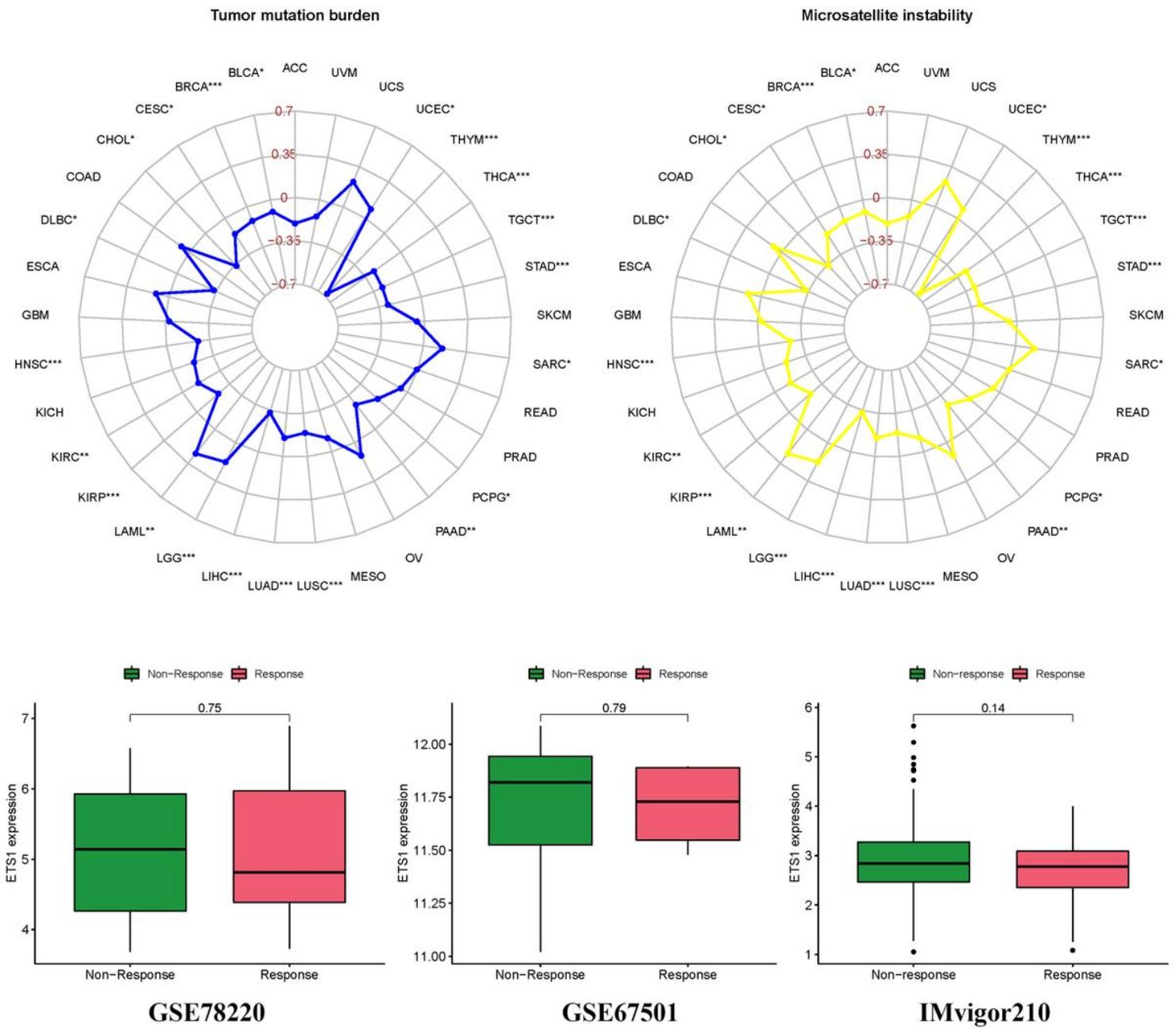


Figure 9

The correlation between ETS1 and both immunotherapeutic markers and response.

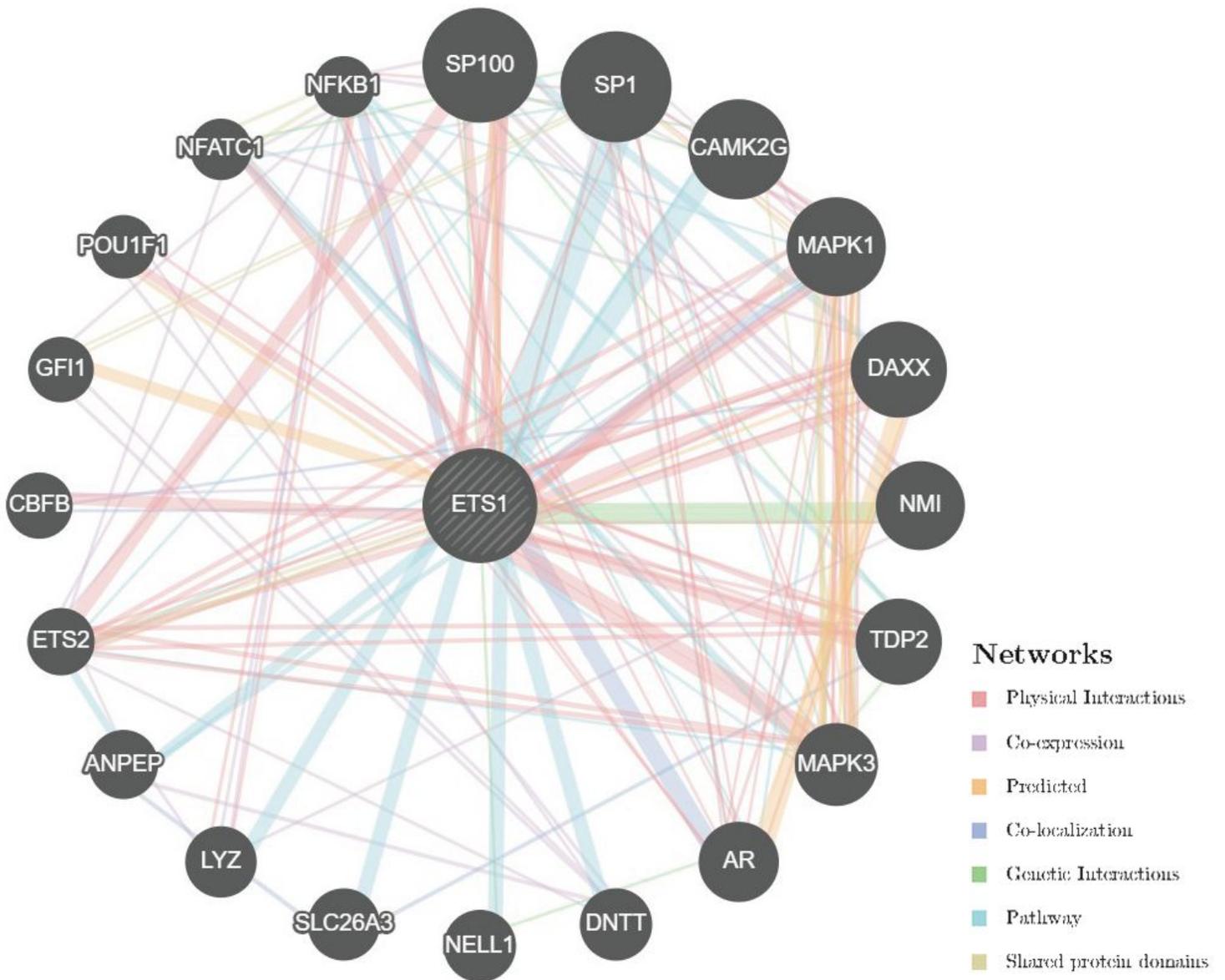


Figure 10

PPI network of ETS1.

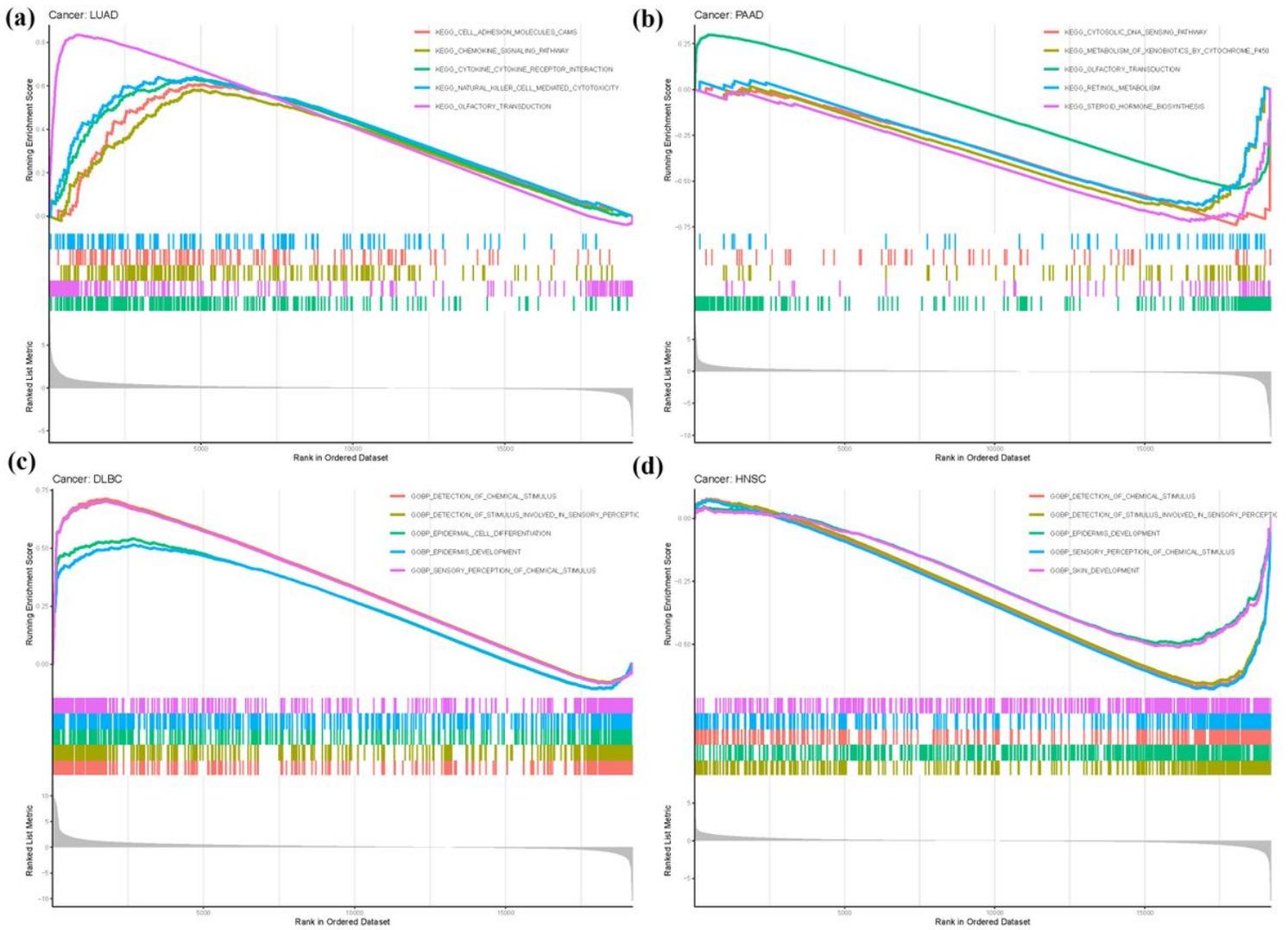


Figure 11

GSEA for samples with ETS1 expression. (a) The low expression. (b) The high expression sample. (c) The low expression. (d) The high expression. (a + b): The enriched gene sets in KEGG. (c + d): The enriched gene sets in GO.