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Comprehensive analysis of selenoprotein gene expression and prognostic value in ovarian cancer

Selenoprotein gen ekspresyonunun ve yumurtalık kanserinde prognostik değerinin kapsamlı analizi

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Abstract

Objective: To comprehensively analyze the expression and prognostic value of selenoprotein in ovarian cancer (OV).

Materials and Methods: GEPIA and cBioPortal were used to analyze selenoprotein expression and mutations and copy number variations. Kaplan-Meier plotter and the tumor immune estimation resource were used to evaluate the impact of these genes on clinical prognosis and their correlation with tumor immune infiltration.

Results: Compared with normal tissues, the expression of iodothyronine deiodinase 3 (DIO3), glutathione peroxidase 4, SECISBP2, SELM, and SELP was decreased in the four gynecological malignancies. In OV, selenoprotein had the highest number of mutations (309) and mutation frequency (52.91%), whereas the lowest was observed in endometrial cancer (29.72%). DIO3, selenoprotein O (SELO), and selenoprotein T (SELT) are significantly related to the prognosis of OV. Immune infiltration analysis showed that DIO3 was associated with tumor-associated macrophages, SELO with $CD4^+$ T-cells and monocytes, and SELT with T-cells. Enrichment analysis revealed that DIO3 is mainly involved in inflammatory immune responses and the Ras signaling pathway, SELO is primarily related to innate immune responses, and SELT is closely associated with mitochondrial oxidative phosphorylation.

Conclusion: This study explored the expression characteristics of 25 selenoprotein in patients with gynecological malignancies and found that DIO3, SELO, and SELT were significantly associated with the prognosis and clinical features of OV, which are potential therapeutic targets.

Keywords: Ovarian cancer, selenoproteins, prognosis, immune infiltration

Öz

Amaç: Yumurtalık kanserinde (YK) selenoproteinlerin ekspresyonunu ve prognostik değerini kapsamlı bir şekilde analiz etmek amaçlanmıştır.

Gereç ve Yöntemler: Selenoprotein ekspresyonunu, mutasyonlarını ve kopya sayısı varyasyonlarını analiz etmek için GEPIA ve cBioPortal kullanıldı. Bu genlerin klinik prognoz üzerindeki etkisini ve tümör immün infiltrasyonu ile korelasyonunu değerlendirmek için Kaplan-Meier plotter ve TIMER kullanıldı.

Bulgular: Normal dokularla karşılaştırıldığında, DIO3, GPX3, SECISBP2, SELM ve SELP ekspresyonları dört jinekolojik malignitede azalmıştır. Yumurtalık kanserinde selenoproteinler en yüksek mutasyon sayısına (309) ve mutasyon sıklığına (%52,91) sahipken, endometriyal kanserde (%29,72) en düşük mutasyon sayısına ve sıklığına sahip idi. DIO3, SELO ve SELT, YK prognozuyla anlamlı olarak ilişkili bulunmuştur. İmmün infiltrasyon analizi, DIO3'ün tümörle ilişkili makrofajlarla, SELO'nun $CD4^+$ T-hücreleri ve monositlerle ve SELT'nin T-hücreleriyle ilişkili olduğunu göstermiştir. Zenginleştirme analizi, DIO3'ün esas olarak enflamatuvar immün yanıtlarda ve Ras sinyal yolunda yer aldığını, SELO'nun esas olarak doğal bağışıklık yanıtlarıyla ilişkili olduğunu ve SELT'in mitokondriyal oksidatif fosforilasyonla yakından ilişkili olduğunu ortaya koymuştur.

Sonuç: Bu çalışmada, jinekolojik malignitelerde 25 selenoproteinin ekspresyon özellikleri araştırılmıştır ve DIO3, SELO ve SELT'in potansiyel terapötik hedef olan YK'nin prognozu ve klinik özellikleriyle önemli ölçüde ilişkili olduğunu bulunmuştur.

Anahtar Kelimeler: Yumurtalık kanseri, selenoproteinler, prognoz, immün infiltrasyon

PRECIS: Iodothyronine deiodinase 3, selenoprotein O, and selenoprotein T were significantly dysregulated in ovarian cancer and associated with the prognosis and clinical features of ovarian cancer, which were potential therapeutic target.

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Introduction

Gynecological malignancies, such as ovarian cancer (OV), cervical cancer, endometrial cancer, and uterine carcinosarcoma, pose significant threats to women's health. With changes in lifestyle and an aging population, the incidence of these tumors has been steadily increasing in China. Among them, cervical cancer has the highest morbidity rate, whereas OV has the highest mortality[1]. However, compared with cervical cancer, effective screening methods for ovarian and endometrial cancers remain inadequate[2]. The challenges of early diagnosis, along with limited treatment options in advanced stages, contribute to the highest mortality rate among all gynecological malignancies. The etiology of gynecological malignancies involves multiple factors, including reproductive history, hormone, genetics, environment, and lifestyle. Therefore, identifying prognostic factors and predictive biomarkers and investigating their underlying mechanisms are crucial for developing more effective diagnostic and therapeutic strategies.

Selenium is a trace element crucial for the biological functions of human cells, particularly in the synthesis of selenoprotein, which possess anti-inflammatory and antioxidant properties[3]. Multiple studies have demonstrated that selenium levels are generally low in most patients with gynecological malignancies and are closely associated with poor prognosis. Additionally, selenium supplementation has been shown to reduce the risk of OV in women[4-8]. A Phase I clinical trial found that using selenium alongside carboplatin and paclitaxel was safe and well tolerated in patients with advanced gynecological malignancies[9]. Mechanistically, higher selenium levels trigger ferroptosis in OV cells by downregulating glutathione peroxidase 4 (GPX4), thereby exerting a therapeutic effect[10]. Despite the potential antitumor effects of selenium, recent epidemiological data indicate that high levels of selenium exposure are associated with an increased incidence of certain cancers[11,12]. 25 selenoprotein have been identified, but their functions have only been partially understood[13]. The hierarchical regulation of selenoprotein in the body and the sex-specific effects of selenium may explain the inconsistent results regarding the effectiveness of selenium supplementation in cancer prevention[14]. Therefore, it is essential to conduct an in-depth exploration of the roles of different selenoprotein in gynecological malignancies, particularly to understand their potential mechanisms and expression patterns.

In this study, we conducted a comprehensive analysis of the expression, mutations, and copy number variations of 25 selenoprotein in patients with gynecological malignancies. Specifically, we focused on OV by performing a prognostic analysis of differentially expressed selenoprotein and further exploring their associations with the clinicopathological characteristics of patients with OV. Through multi-omics data analysis, we identified that iodothyronine deiodinase 3 (DIO3), selenoprotein O (SELO), and selenoprotein T (SELT) are significantly dysregulated in OV and are associated with poor prognosis. Additionally, we conducted immune infiltration analysis and gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses on these selenoprotein-related genes to explore their potential biological functions and mechanisms in OV. Therefore, this study enhances our understanding of the potential roles of selenoprotein in the initiation and progression of OV.

Materials and Methods

Analysis of selenoprotein mRNA expression

The Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) database was used to analyze the difference in the mRNA expression of selenoprotein between tumor and normal tissues and to investigate the correlation between selenoprotein gene expression and immune cell marker genes in OV[15].

Analysis of selenoprotein mutations and copy number variations

The cancer genome atlas (TCGA)-OV, cervical cancer, endometrial cancer, and uterine carcinosarcoma datasets from the cBioPortal (http://www.cbioportal.org/) database were used to perform mutation and copy number variation analyses of selenoprotein, as well as prognosis analysis before and after gene mutations[16,17].

Analysis of Kaplan-Meier Plotter database

Kaplan-Meier plotter (http://kmplot.com/analysis/) database was used to analyze the correlation between selenoprotein expression and the survival of OV patients[18].

Protein expression analysis of selenoprotein

Immunohistochemical images of SELO and SELT were obtained from the Human protein mapping (HPA, https://www.proteinatlas.org/) database. The Universal Analysis of Cancer (UALCAN, http://ualcan.path.uab.edu/) database was used to obtain expression data for DIO3, SELO, and SELT based on various clinical characteristics of OV[19].

Immune infiltration analysis

The tumor immune estimation resource (TIMER, https://cistrome.shinyapps.io/timer/) database was used to assess the association between DIO3, SELO, and SELT with tumor-infiltrating immune cells and immune cell marker genes[20].

Gene correlation and enrichment analysis

WebGestalt (https://www.webgestalt.org/) was used to perform GO and KEGG pathway enrichment analyses of genes correlated with DIO3, SLEO, and selenoprotein T (SLET), which were obtained from LinkedOmics (http://linkedomics.org/login.php)[21,22].

Statistical Analysis

Survival curves were generated using the Kaplan-Meier plotter, and the results are presented as hazard ratios and p-values

derived from the logrank test. Spearman's exact test was used to analyze the correlation between gene expression. The bubble map is plotted using the R ggplot package. P<0.05 was considered statistically significant.

Results

mRNA expression of selenoprotein in different types of gynecological malignancies

First, the results from the GEPIA database showed that in four gynecological malignancies, the expression levels of DIO3, GPX3, SECISBP2, SELM, and SELP were generally lower than those in normal tissues (Figure 1). However, DIO1, EEFSEC, SELI, SELK, SELV, SELW, SEPHS1, TXNRD1, and TXNRD2 were not dysregulated (Supplementary Figure 1). Additionally, some selenoprotein exhibit significant changes in expression in specific tumor types. Compared with normal tissues, GPX1, SLET, and SEPHS2 expression was significantly increased in OV, whereas SEPSECS, TXNRD3, and SELO expression was significantly downregulated. In cervical cancer, GPX2 and MSRB1 were markedly upregulated, whereas SELENBP1 was significantly downregulated. In endometrial cancer, the expression of DIO2, GPX1, GPX4, and SEPHS2 was notably increased, whereas SEPSECS showed a significant decrease in uterine carcinosarcoma (Supplementary Figure 2, 3).

Mutations and copy number variations of selenoprotein in TCGA-OV, cervical cancer, endometrial cancer, and uterine carcinosarcoma datasets

Next, we found that OV had the highest number of selenoprotein gene mutations (309) and the highest mutation frequency (52.91%), whereas endometrial cancer had the fewest mutations (162) and a frequency of 29.72% (Figure 2A). Additionally, patients with OV mutations showed better overall survival compared with those without mutations (p=0.0347), but there was no significant difference in disease-free survival (p=0.734) (Figure 2B, C). In contrast, compared with the non-mutated group, patients with mutations in endometrial cancer had worse overall survival (p=0.151) and disease-free survival (p=0.0902). For cervical cancer and uterine carcinosarcoma, there were no significant differences in OS and disease-free survival between the mutated and non-mutated groups

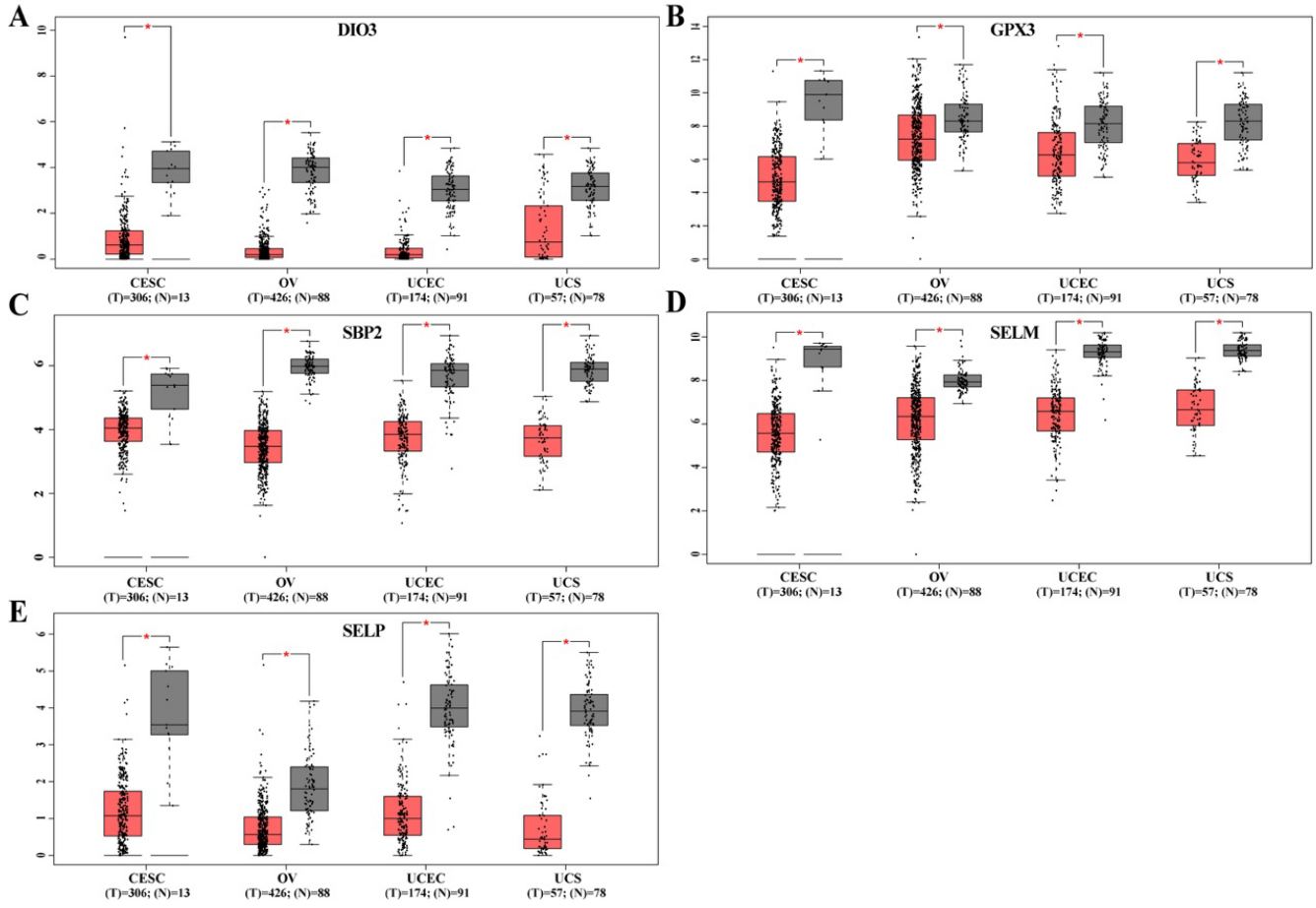

Figure 1. The mRNA expression of selenoprotein in four gynecological malignancies (GEPIA). A-E) The expression of five consistently downregulated selenoprotein in tumor and normal tissues [DIO3 (A), GPX3 (B), SECISBP2 (C), SELM (D), and SELP (E)]

The red asterisks (*) indicating significant differences (p<0.05), CESC: Cell carcinoma and endocervical adenocarcinoma, OV: Ovarian cancer, UCEC: Uterine corpus endometrial carcinoma

(Supplementary Figure 4A-C). Moreover, SELT (15%), SELV (12%), and SELENBP1 (12%) had the highest mutation rates in OV; SELT (8%) and SELP (7%) had the highest mutation rates in cervical cancer; SELENBP1 (10%) and SELT (5%) were the most frequently mutated in endometrial cancer; SELT (21%) and SELENBP1 (13%) showed the highest mutation rates in uterine carcinosarcoma (Figure 2D and Supplementary Figure 4D-F). Based on these results, we selected OV as the focus of our subsequent research.

Prognostic value of selenoprotein in OV

For the prognostic value of differentially expressed selenoprotein in OV, we found that low expression of DIO3, SECISBP2, and SELO, as well as high expression of GPX3, SELM, and SELP, were associated with poorer overall survival (Figure 3A). Additionally, low expression of DIO3, SELO, and SEPHS2 and high expression of GPX3, SECISBP2, SELM, and SELT were associated with worse progression-free survival (Figure 3B). Based on the expression differences and clinical significance of these genes, we selected DIO3, SELO, and SELT as the primary molecules for further research.

Association between DIO3, SELO, and SELT expression and clinicopathological features in patients with OV

By analysis in the HPA, UALCAN, and Kaplan-Meier plotter databases, we found that DIO3, SELO, and SELT were not significantly correlated with the clinical stages or tumor grades of OV. However, as the tumor grade increased (indicating lower

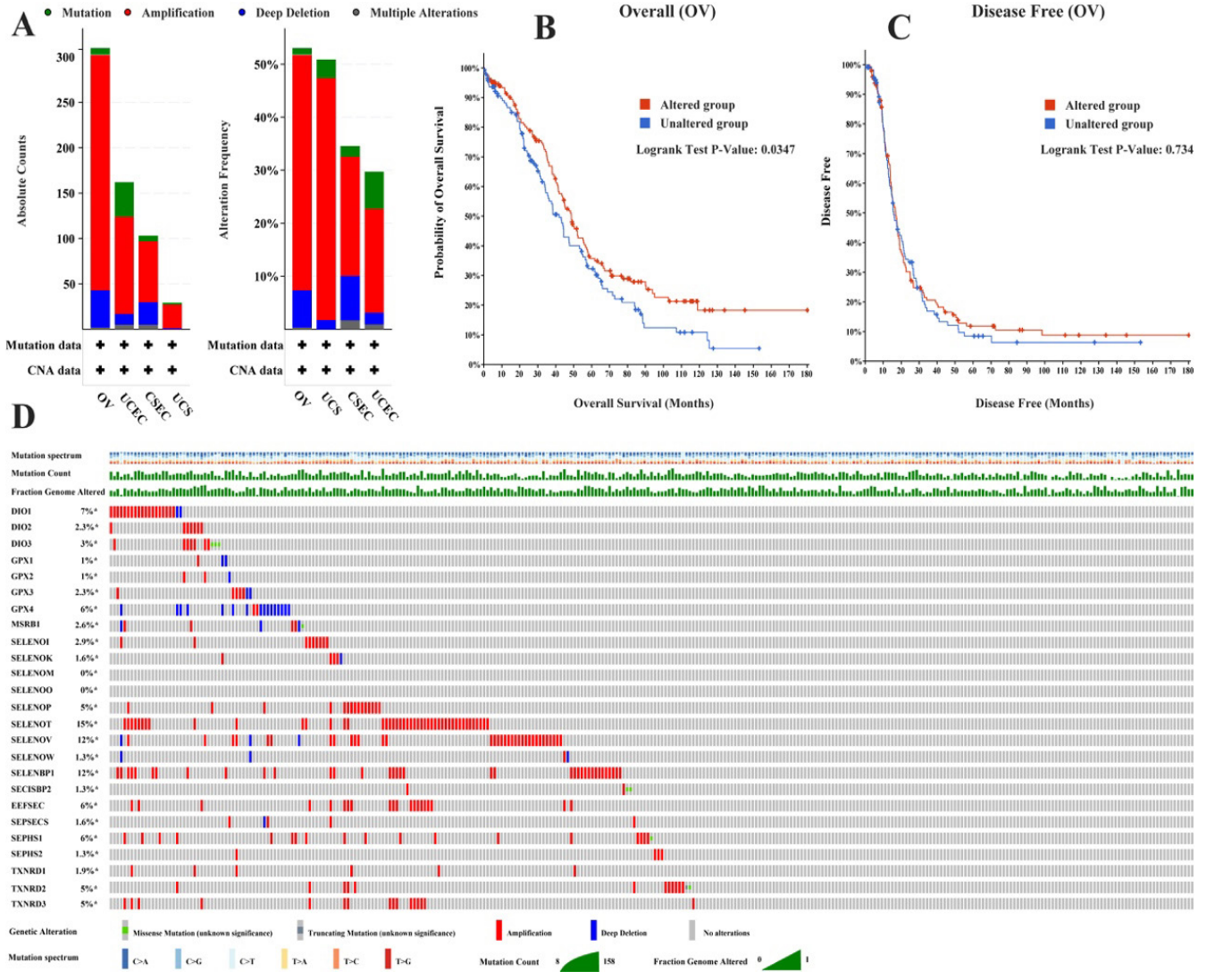

Figure 2. Mutations and copy number variations of selenoprotein (cBioPortal). Numbers and frequencies of selenoprotein mutations in four gynecological malignancies. B, C. Comparison of overall survival (B) and disease-free survival (C) between the selenoprotein gene-mutated and non-mutated groups in patients with OV. Relationship between mutations and copy number variations of 25 selenioproteins and OV

OV: Ovarian cancer, Logrank p<0.05 indicates statistical significance, CESC: Cell carcinoma and endocervical adenocarcinoma, UCEC: Uterine corpus endometrial carcinoma

differentiation), the expression of DIO3 tended to decrease gradually (Figure 4A, B). Regarding the TP53 mutation status, unlike DIO3 and SELO, SELT expression was significantly increased in patients with TP53 mutations (Figure 4C). At the protein level (data on DIO3 is lacking), immunohistochemistry and total protein analysis revealed that SELO expression was significantly reduced, whereas SELT expression was significantly increased in OV. SELO protein levels decreased with advancing tumor stage and grade, whereas SELT protein levels increased in patients with higher stages (stages 2 and 3) and grades (grades 2 and 3) (Figure 4D-G). Low DIO3 expression was significantly associated with poorer overall survival and progression-free survival in OV patients with the following characteristics: CA125 levels below the lower quartile, optimal or suboptimal debulk, and receiving platinum-based chemotherapy (Supplementary Table 1, p<0.05). Additionally, low expression of SLEO was also significantly associated with poorer overall survival and progression-free survival in serous and grade 2-3 OV patients, which may also exhibit average CA125 levels below the lower quartile and optimal debulk (Supplementary Table 2, p<0.05). High SELT expression was significantly associated with poorer progression-free survival in the following patient groups: Serous OV, stage 3-4, grade 3, P53 mutation, optimal debulk, and receiving platinum-based or gemcitabine chemotherapy (Supplementary Table 3, p<0.05).

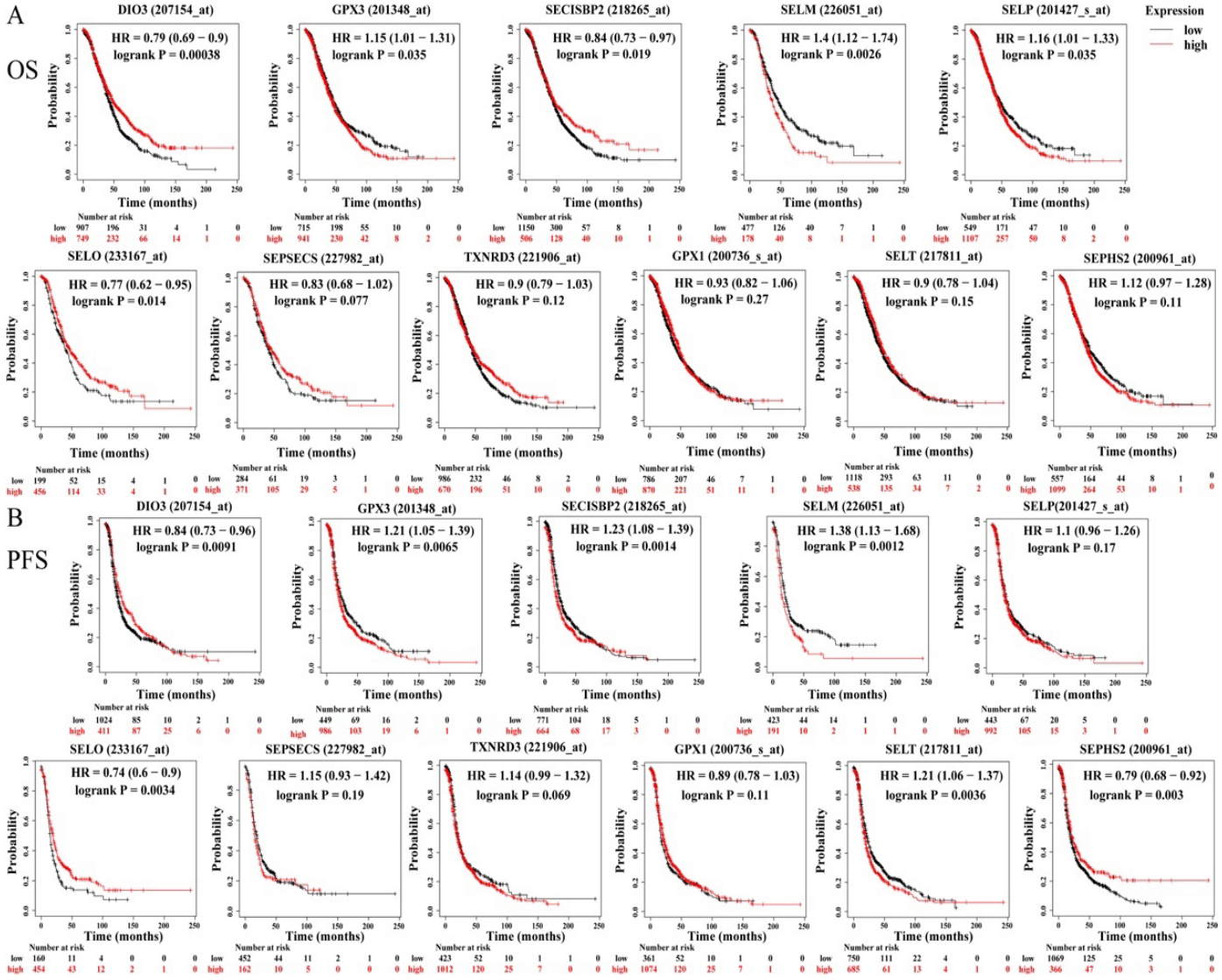

Figure 3. Prognostic value of selenoprotein differentially expressed in OVs (Kaplan-Meier plotter). A-B. Comparison of overall survival (A) and progression-free survival (B) between the high and low selenoprotein expression groups in patients with ovarian cancer

OV: Ovarian cancer, DIO3: Iodothyronine deiodinase 3, SELO: Selenoprotein O, SELT: Selenoprotein T, HR: Hazard ratio. Logrank p<0.05 indicates statistical significance

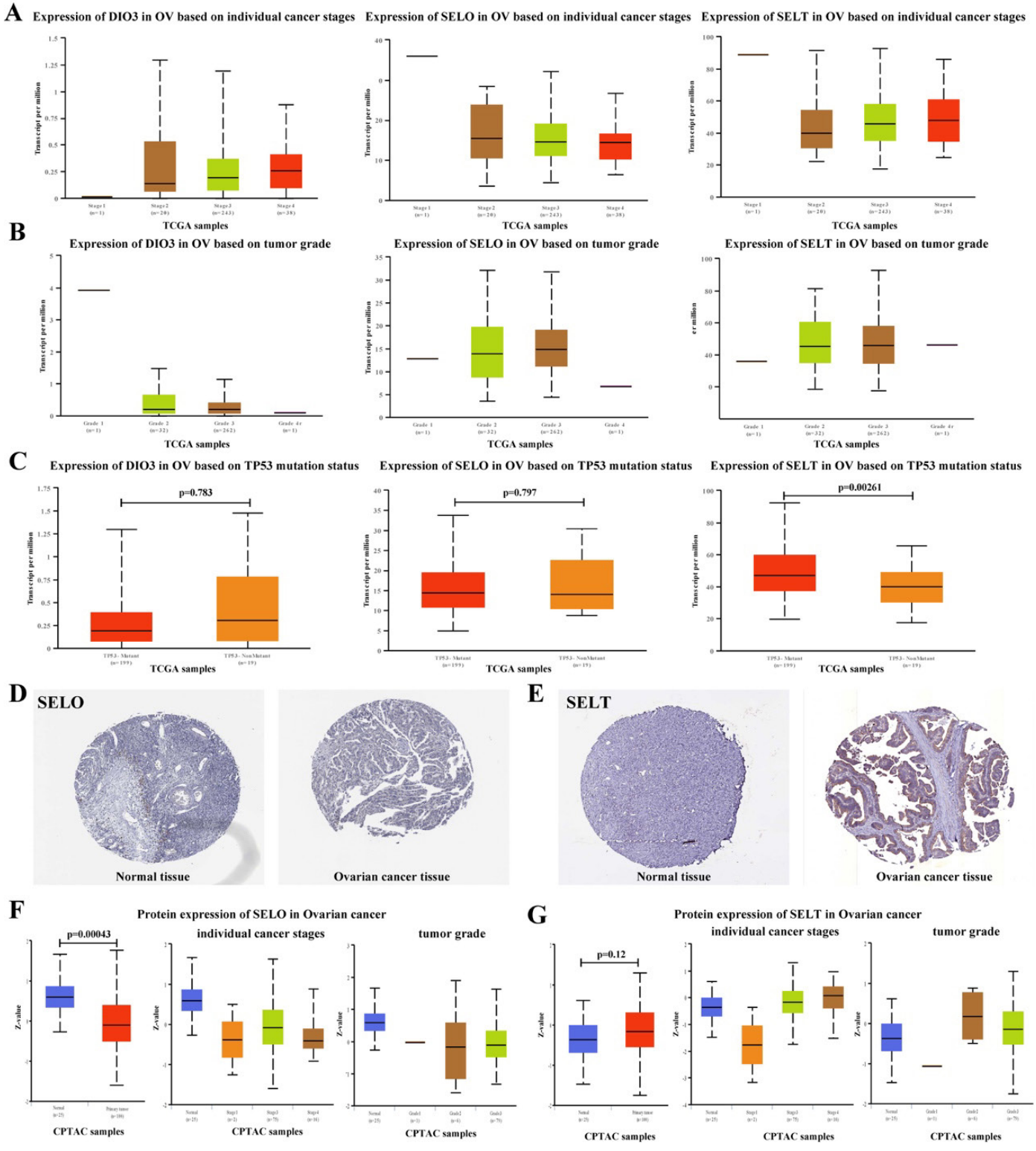

Figure 4. Association between DIO3, SELO, and SELT expression and clinicopathological features in patients with OV (UALCAN and HPA). A-C) DIO3, SELO, and SELT expression in OV based on tumor stage (A), tumor grade (B), and TP53 mutation (C). D, E) Representative immunohistochemical images of SELO (D) and SELT € in normal and OV tissues. Protein levels of SLEO (F) and SELT (G) in normal and ovarian cancer tissues based on tumor stage and grade

OV: Ovarian cancer, DIO3: Iodothyronine deiodinase 3, SELO: Selenoprotein O, SELT: Selenoprotein T, UALCAN: Universal Analysis of Cancer, HPA: Human protein mapping, TCGA: Cancer genome atlas, CPTAC: Clinical proteomic tumor analysis consortium. P<0.05 was considered statistically significant

Association between DIO3, SELO, and SELT expression and immune infiltration in OV

Tumor-infiltrating lymphocytes are independent predictors of cancer survival. We found that DIO3 was negatively associated with macrophage infiltration (r=-0.165, p=2.89e^{-4}) (Figure 5A); SELO was negatively correlated with tumor purity (r=-0.159, p=0.0117), while positively correlated with CD4$^+$ T-cells (r=0.168, p=0.0084) (Figure 5B); SELT was negatively correlated with tumor purity (r=-0.158, p=4.78e^{-4}), while positively associated with CD8$^+$ T-cells (r=0.15, p=9.73e^{-4}), CD4$^+$T-cells (r=0.094, p=0.0403), macrophages (r=0.277, p=7.04e^{-10}), neutrophils (r=0.285, p=1.99e^{-10}) and dendritic cells (r=0.201, p=9.35e^{-6}) (Figure 5C).

Correlation analysis of DIO3, SELO, and SELT expression with immune cell marker genes

The analyses in the TIMER and GEPIA databases revealed that DIO3 was significantly correlated with tumor-associated macrophage (TAM) marker genes and some marker genes of different T-cell subsets in OVs. SELO was mainly significantly associated with the marker genes of CD4$^+$ T-cells and monocytes. Moreover, SELT was significantly correlated with the marker genes of total T-cell, CD8$^+$ T-cell, Th1 cell, and exhausted T-cell (Supplementary Table 4, 5). Specifically, *CCL2, CD68*, and *IL10* (TAMs marker genes) were significantly correlated with DIO3 (Figure 5D). *CD4* (CD4$^+$ T-cell marker gene) and *CD86* and CSF1R (monocyte marker genes) were significantly correlated with SELO (Figure 5E). *CD2, CD3D* (total T-cell marker genes), *CD8B* (CD8$^+$ T-cell marker genes), *STAT1, STAT4, IFNG, TNF* (Th1 cell marker genes), *PDCD1, CTLA4, LAG3,* and *HAVCR2* (exhausted T-cell marker genes) were significantly correlated with SELT (Figure 5F).

Biological functions and signaling pathways of DIO3, SELO, and SELT in OV

Significantly correlated genes with DIO3, SELO, and SELT in OV were identified by LinkedOmics database. The expression patterns of the top 50 positively and negatively correlated genes are presented as heatmaps (Supplementary Figure 5A-C). GO enrichment analysis indicated that DIO3 was positively associated with inflammatory and immune responses and Ras activity. In contrast, it was mainly negatively correlated with cilium assembly and microtubule movement (Supplementary Figure 6A). SELO was mainly involved in NF-κB signaling pathway and MAP kinase activity, while negatively regulating chromatin and histone modification (Supplementary Figure 6B). SELT was associated with mitochondria-related biological activities and also showed a negative correlation with the regulation of chromatin and histones (Supplementary Figure 6C). Additionally, KEGG enrichment analysis revealed that DIO3 was primarily involved in Ras and chemokine signaling pathways (Figure 6A). SELO was mainly associated with the NOD-like receptor, toll-like receptor, and TNF signaling pathways (Figure 6B). Consistent with the GO enrichment analysis, SELT was closely related to oxidative phosphorylation (Figure 6C).

Discussion

Limited treatment options, resistance to existing chemotherapeutic drugs, and tumor recurrence are the primary obstacles to extending the survival of patients with gynecological malignancies. Selenium, an essential trace element, has significant antiviral properties and antitumor effects. Although clinical trials on selenium supplementation for the prevention of endometrial and cervical cancers have yielded mixed results, studies suggest that selenium may reduce the risk of developing OV[5,23,24]. Additionally, selenium supplements have been shown to significantly alleviate the toxic side effects associated with chemotherapy or radiotherapy, thereby improving the quality of life of patients[24-26]. Selenium exerts its effects in the body primarily through the synthesis of selenoprotein, which have anti-inflammatory and antioxidant properties. However, the specific functions and mechanisms of most selenoprotein remain unclear. Therefore, this study comprehensively analyzed the expression patterns of 25 selenoprotein in gynecological malignancies and their potential prognostic value in OV, aiming to provide a scientific basis for the application of selenoprotein in cancer therapy.

Several selenoprotein, such as DIO3, GPX3, SECISBP2, SELM, and SELP, are significantly downregulated in gynecological malignancies, which may be related to lower serum selenium levels in patients with cancer[27,28]. By analyzing the selenoprotein mutations, we observed these genes exhibit the highest number and frequency of mutations in OV. Moreover, patients with OV with mutations in these genes had a significantly higher tumor mutational burden (TMB) compared with those without mutations, which is often associated with better overall survival. It has been reported that TMB levels are significantly positively correlated with the effectiveness of PD-1 inhibitors, and patients with tumors with high TMB levels lived longer[29]. This may be because a higher number of mutated genes leads to the production of more abnormal proteins, thereby enhancing the recognition and activation of the immune system, which in turn improves the effectiveness of immunotherapy and chemotherapy. Through prognostic and immune infiltration analyses, we found that the expression of DIO3, SELO, and SELT was significantly associated with the prognosis and clinical characteristics of patients undergoing OV. These genes are also involved in regulating the infiltration of immune cells into the tumor microenvironment. These findings revealed the important roles of DIO3, SELO, and SELT in the pathogenesis of OV and may provide new targets for future therapeutic strategies.

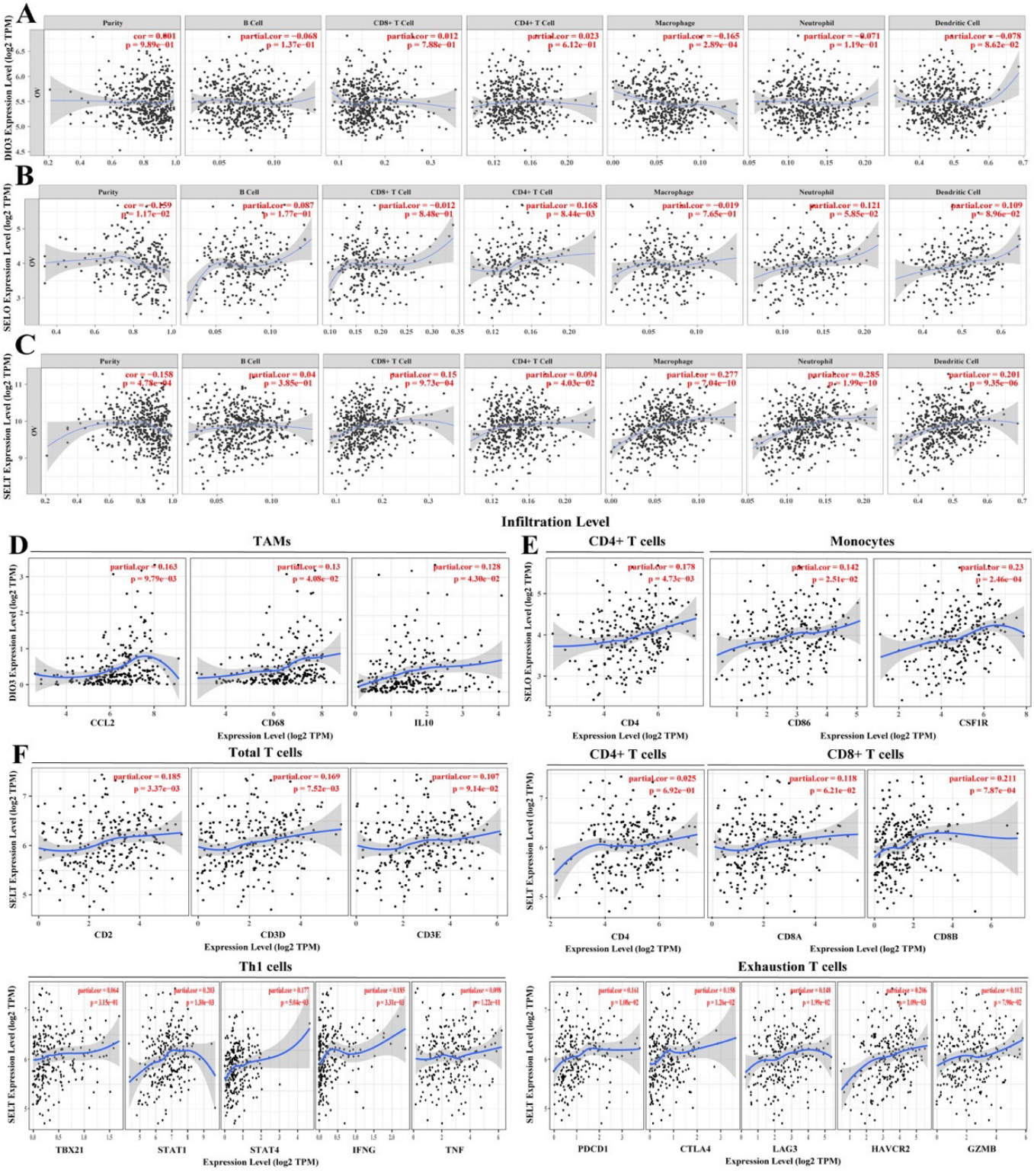

Figure 5. Correlation between the expression of DIO3, SELO, and SELT and immune infiltration in ovarian cancer (TIMER). Correlation between the expression of DIO3 (A), SELO (B), and SELT (C) and tumor purity, as well as the infiltration of B-cells, CD8+ T-cells, CD4+ T-cells, macrophages, neutrophils, and dendritic cells. Correlations between DIO3 expression, CCL2CD68 expression, and IL10. E. Correlations between SELO expression and CD4, CD86, and CSF1R expression. Correlation between SELT expression and CD2CD3D, CD3E, CD8A, CD8B, TBX21, STAT1, STAT4, IFNG, TNF, PDCD1, CTLA4, LAG3, HAVCR2, GZMB. P<0.05 was considered statistically significant

DIO3: Iodothyronine deiodinase 3, SELO: Selenoprotein O, SELT: Selenoprotein T, TNF: Tumor necrosis factor, TIMER: Tumor immune estimation resource

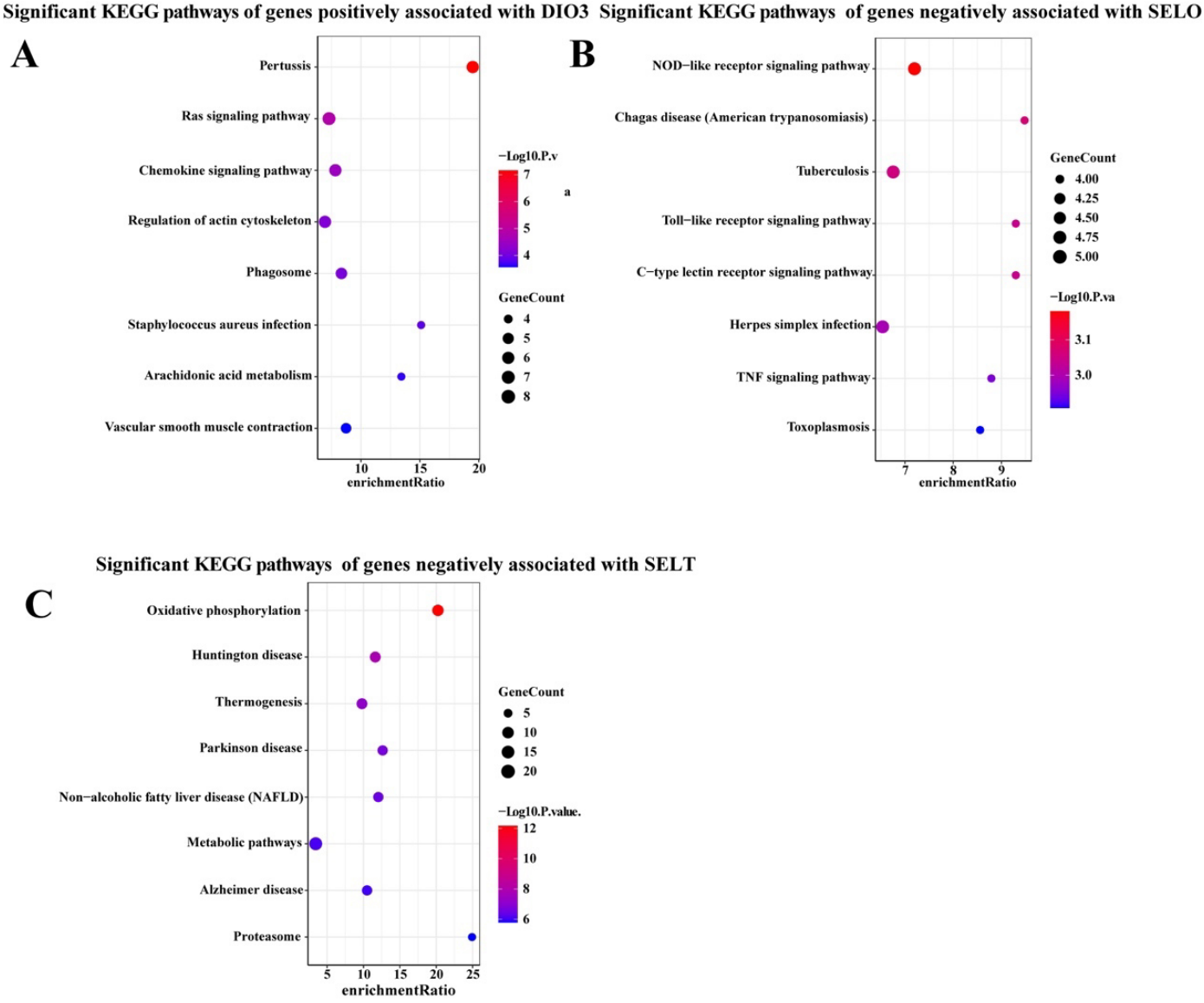

Figure 6. KEGG enrichment analysis of genes associated with DIO3, SELO, and SELT in ovarian cancer (LinkedOmics and WebGestalt). A-C. Bubble plot showing the KEGG enrichment analysis results for the top 100 genes with significant positive/negative associations with DIO3 (A), SELO (B), and SELT (C)

KEGG: Kyoto Encyclopedia of Genes and Genomes, DIO3: Iodothyronine deiodinase 3, SELO: Selenoprotein O, SELT: Selenoprotein T, p<0.05 was considered statistically significant

DIO3 contains a selenocysteine (Sec) active site that is capable of inactivating thyroid hormone T3. In various tumor types, abnormal DIO3 expression is closely associated with tumor proliferation and differentiation[30]. Our findings suggest that DIO3 is a potential biomarker and therapeutic target of OV. In contrast, Moskovich et al.[31,32] found that increased DIO3 expression promoted tumor development and metabolic reprograming by modulating T3 in high-grade serous OV. They further found that a small-molecule inhibitor targeting DIO3 was effective in inhibiting tumor growth[31,32]. However, low DIO3 expression was negatively associated with overall survival and progression-free survival in low-grade (grade 1) OV. In high-grade (grade 2+3 and grade 3) OV, low DIO3 expression was still significantly negatively associated with overall survival but positively associated with progression-free survival (Supplementary Table 1). These results suggest that DIO3 may play a very different role in different types, stages, and grades of OV. In addition to regulating the deactivation of T3, DIO3 may also be involved in epigenetic regulation through genomic imprinted regions co-formed with DLK1[33]. Frequent interactions between different regulatory pathways may contribute to contradictory findings. Consistent with our

immune infiltration and enrichment analyses, Zhang et al.[34] demonstrated that the DLK1-DIO3 locus is closely linked to Ras-induced hepatocarcinogenesis. Additionally, the DLK1-DIO3 region has been associated with alterations in immune cell and inflammatory cytokine levels in various diseases[35-37]. However, reports on DLK1-DIO3 in OV are extremely limited, indicating the urgent need for further research to explore the potential role of DIO3 in OV.

SELO is a mitochondrial protein with redox activity involved in ATP amidation[38,39]. Previous studies have shown that SELO is downregulated in gastric and liver cancers, and this downregulation is associated with poor prognosis in patients[40,41]. Similarly, SELO was significantly downregulated in four gynecological malignancies. The multi-omics analysis further revealed that low SELO expression is associated with poor prognosis in patients undergoing OV. This effect may be mediated through the regulation of innate immune response pathways, which influence the dynamics of tumor burden and the infiltration of $CD4^+$ T-cells. However, no studies have investigated the involvement of SELO in tumor pathogenesis or immune regulation processes. Therefore, further investigation into SELO's regulatory role in OV, particularly through adaptive immune response pathways and its redox activity, represents a novel and significant research direction.

SELT is an endoplasmic reticulum membrane protein with thioredoxin reductase activity[42]. Studies have shown that SELT expression is significantly increased in breast cancer, and it contributes to the prevention of apoptosis in cancer cells[43]. Additionally, SELT protects the heart from ischemia-reperfusion injury by inhibiting apoptosis and oxidative stress[44]. In this study, we found that SELT expression was significantly increased in Ovs and was closely associated with poor patient prognosis and resistance to platinum-based chemotherapy. This may be because SELT protected OV cells against apoptosis by inhibiting oxidative stress responses and calcium ion flux, thereby promoting tumor growth. Furthermore, our results suggest that SELT plays a critical role in T-cell differentiation and homeostasis regulation through oxidative phosphorylation. The differentiation of T-cells is closely linked to changes in energy metabolism: Naive and memory T-cells maintain high levels of oxidative phosphorylation, whereas effector T-cells rely on aerobic glycolysis. In contrast, continuous tumor antigen stimulation could impair the oxidative phosphorylation pathway in activated T-cells, leading to their transition into hypometabolic exhausted T-cells, which suppresses both mitochondrial respiration and glycolytic function[45]. Although no direct studies have linked SELT to T-cell differentiation, SELT-regulated oxidative phosphorylation and mitochondrial respiration may play critical roles in the remodeling of the tumor immune microenvironment in OV. Future research should focus on the relationship between SELT-regulated tumor immune microenvironment and OV progression.

Study Limitations

This study utilized multiple databases to validate the reliability of the findings. However, there are some limitations. Further fundamental experiments are essential to elucidate the molecular mechanisms of selenoprotein in the progression of OV.

Conclusion

In conclusion, through comprehensive bioinformatics analysis, this study revealed an association between dysregulated expression of DIO3, SELO, and SELT and poor prognosis in OV. We further explored the functions and pathways involved in these three selenoprotein to elucidate their roles in disease development in OV. Our findings not only provide new insights into the possible regulatory pathways of DIO3, SELO, and SELT but also provide new perspectives on the role of these selenoprotein in OV.

Ethics

Ethics Committee Approval: The Yuhang Third People's Hospital Ethics Committee has confirmed that no ethics approval was required, and this study was performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000.

Informed Consent:

Acknowledgments

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Footnotes

Authorship Contributions

Concept: Y.H., H.D., Design: Y.H., H.D., Data Collection or Processing: Y.H., H.S., H.D., Analysis or Interpretation: Y.H., H.D., Literature Search: Y.H., H.S., H.D., Writing: Y.H.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Xia C, Dong X, Li H, Cao M, Sun D, He S, et al. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. Chin Med J (Engl). 2022;135:584-90.
2. Zhou Z, Li W, Zhang F, Hu K. The value of squamous cell carcinoma antigen (SCCa) to determine the lymph nodal metastasis in cervical cancer: A meta-analysis and literature review. PLoS One. 2017;12:e018616.
3. Rayman MP. Selenium and human health. Lancet. 2012;379:1256-68.
4. Sundström H, Ylikorkala O, Kauppila A. Serum selenium and thromboxane levels in patients with gynecological cancer. Carcinogenesis. 1986;7:1051-2.
5. Terry PD, Qin B, Camacho F, Moorman PG, Alberg AJ, Barnholtz-Sloan JS, et al. Supplemental selenium can decrease ovarian cancer risk in African-American women. J Nutr. 2017;147:621-7.

6. Gifkins D, Olson SH, Paddock L, King M, Demissie K, Lu SE, et al. Total and individual antioxidant intake and risk of epithelial ovarian cancer. BMC Cancer. 2012;12:211.
7. Janowska M, Potocka N, Paszek S, Skrzypa M, Wróbel A, Kluz M, et al. Serum selenium concentration in women with endometrial cancer. Nutrients. 2022;14:958.
8. Qi L, Wang Y, Wang R, Wang M, Jablonska E, Zhou H, et al. Association between plasma selenium and its untargeted metabolomic profiling and cervical cancer prognosis. Biol Trace Elem Res . 2023;201:4637-48.
9. Song M, Kumaran MN, Gounder M, Gibbon DG, Nieves-Neira W, Vaidya A, et al. Phase I trial of selenium plus chemotherapy in gynecologic cancers. Gynecol Oncol. 2018;150:478-86.
10. Choi JA, Lee EH, Cho H, Kim JH. High-dose selenium induces ferroptotic cell death in ovarian cancer. Int J Mol Sci. 2023;24:1918.
11. Vinceti M, Vicentini M, Wise LA, Sacchettini C, Malagoli C, Ballotari P, et al. Cancer incidence following long-term consumption of water with high inorganic selenium content. Sci Total Environ. 2018;635:390-6.
12. Narod SA, Huzarski T, Jakubowska A, Gronwald J, Cybulski C, Oszurek O, et al. Serum selenium levels and cancer risk: a nested case–control study. Hered Cancer Clin Pract. 2019;17:33.
13. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigó R, et al. Characterization of mammalian selenoproteomes. Science. 2003;300:1439-43.
14. Schomburg L, Schweizer U. Hierarchical regulation of selenoprotein expression and sex-specific effects of selenium. Biochim Biophys Acta. 2009;1790:1453-62.
15. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45:W98-102.
16. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2:401-4. Erratum in: Cancer Discov. 2012;2:960.
17. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6:pl1.
18. Lánczky A, Győrffy B. Web-based survival analysis tool tailored for medical research (KMplot): Development and implementation. J Med Internet Res. 2021;23:e27633.
19. Chandrashekar DS, Karthikeyan SK, Korla PK, Patel H, Shovon AR, Athar M, et al. UALCAN: An update to the integrated cancer data analysis platform. Neoplasia. 2022;25:18-27.
20. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77:e108-e10.
21. Vasaikar SV, Straub P, Wang J, Zhang B. LinkedOmics: analysis of multi-omics data within and across 32 cancer types. Nucleic Acids Res. 2018;46:D956-63.
22. Liao Y, Wang J, Jaehnig EJ, Shi Z, Zhang B. WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. Nucleic Acids Res. 2019;47:W199-205.
23. 23. Kho PF, Glubb DM, Thompson DJ, Spurdle AB, O'Mara TA. The Role of Selenium in endometrial cancer risk: A mendelian randomization study. Front Oncol. 2019;9:182.
24. Muecke R, Micke O, Schomburg L, Glatzel M, Reichl B, Kisters K, et al. Multicenter, phase III trial comparing selenium supplementation with observation in gynecologic radiation oncology: follow-up analysis of the survival data 6 years after cessation of randomization. Integr Cancer Ther. 2014;13:463-7.
25. Muecke R, Schomburg L, Glatzel M, Berndt-Skorka R, Baaske D, Reichl B, et al. Multicenter, phase 3 trial comparing selenium supplementation with observation in gynecologic radiation oncology. Int J Radiat Oncol Biol Phys. 2010;78:828-35.
26. Sieja K, Talerczyk M. Selenium as an element in the treatment of ovarian cancer in women receiving chemotherapy. Gynecol Oncol. 2004;93:320-7.
27. Kluza M, Paszek S, Kluza K, Januszek S, Potocka N, Skrzypa M, et al. Serum selenium concentration in women with ovarian cancer. Nutrients. 2023;15:850.
28. He D, Wang Z, Huang C, Fang X, Chen D. Serum selenium levels and cervical cancer: A systematic review and meta-analysis. Biol Trace Elem Res. 2017;179:195-202.
29. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Ann Oncol. 2019;30:44-56.
30. Luongo C, Dentice M, Salvatore D. Deiodinases and their intricate role in thyroid hormone homeostasis. Nat Rev Endocrinol. 2019;15:479-88.
31. Moskovich D, Alfandari A, Finkelshtein Y, Weisz A, Katzav A, Kidron D, et al. DIO3, the thyroid hormone inactivating enzyme, promotes tumorigenesis and metabolic reprograming in high-grade serous ovarian cancer. Cancer Lett. 2021;501:224-233. Erratum in: Cancer Lett. 2023;563:216196.
32. Moskovich D, Finkelshtein Y, Alfandari A, Rosemarin A, Lifschytz T, Weisz A, et al. Targeting the DIO3 enzyme using first-in-class inhibitors effectively suppresses tumor growth: a new paradigm in ovarian cancer treatment. Oncogene. 2021;40:6248-57. Erratum in: Oncogene. 2023;42:1508.
33. Glazov EA, McWilliam S, Barris WC, Dalrymple BP. Origin, evolution, and biological role of miRNA cluster in DLK-DIO3 genomic region in placental mammals. Mol Biol Evol. 2008;25:939-48.
34. Zhang J, Li H, Dong J, Zhang N, Liu Y, Luo X, et al. Omics-based identification of shared and gender disparity routes in *Hras12V*-induced hepatocarcinogenesis: An important role for Dlk1-Dio3 genomic imprinting region. Front Genet. 2021;12:620594.
35. Groeneveld S, Faget J, Zangger N, Meylan E. Snail mediates repression of the Dlk1-Dio3 locus in lung tumor-infiltrating immune cells. Oncotarget. 2018;9:32331-45.
36. Al-Harbi S, Choudhary GS, Ebron JS, Hill BT, Vivekanathan N, Ting AH, et al. miR-377-dependent BCL-xL regulation drives chemotherapeutic resistance in B-cell lymphoid malignancies. Mol Cancer. 2015;14:185.
37. Dai R, Lu R, Ahmed SA. The upregulation of genomically imprinted DLK1-Dio3 miRNAs in Murine Lupus Is Associated with Global DNA Hypomethylation. PLoS One. 2016;11:e0153509.
38. Han SJ, Lee BC, Yim SH, Gladyshev VN, Lee SR. Characterization of mammalian selenoprotein o: a redox-active mitochondrial protein. PLoS One. 2014;9:e95518.
39. Sreelatha A, Yee SS, Lopez VA, Park BC, Kinch LN, Pilch S, et al. Protein AMPylation by an Evolutionarily Conserved Pseudokinase. Cell. 2018;175:809-21.e19.

40. Lan X, Xing J, Gao H, Li S, Quan L, Jiang Y, et al. Decreased selenoprotein expression as a poor prognosticator of gastric cancer in humans. Biol Trace Elem Res. 2017;178:22-8.

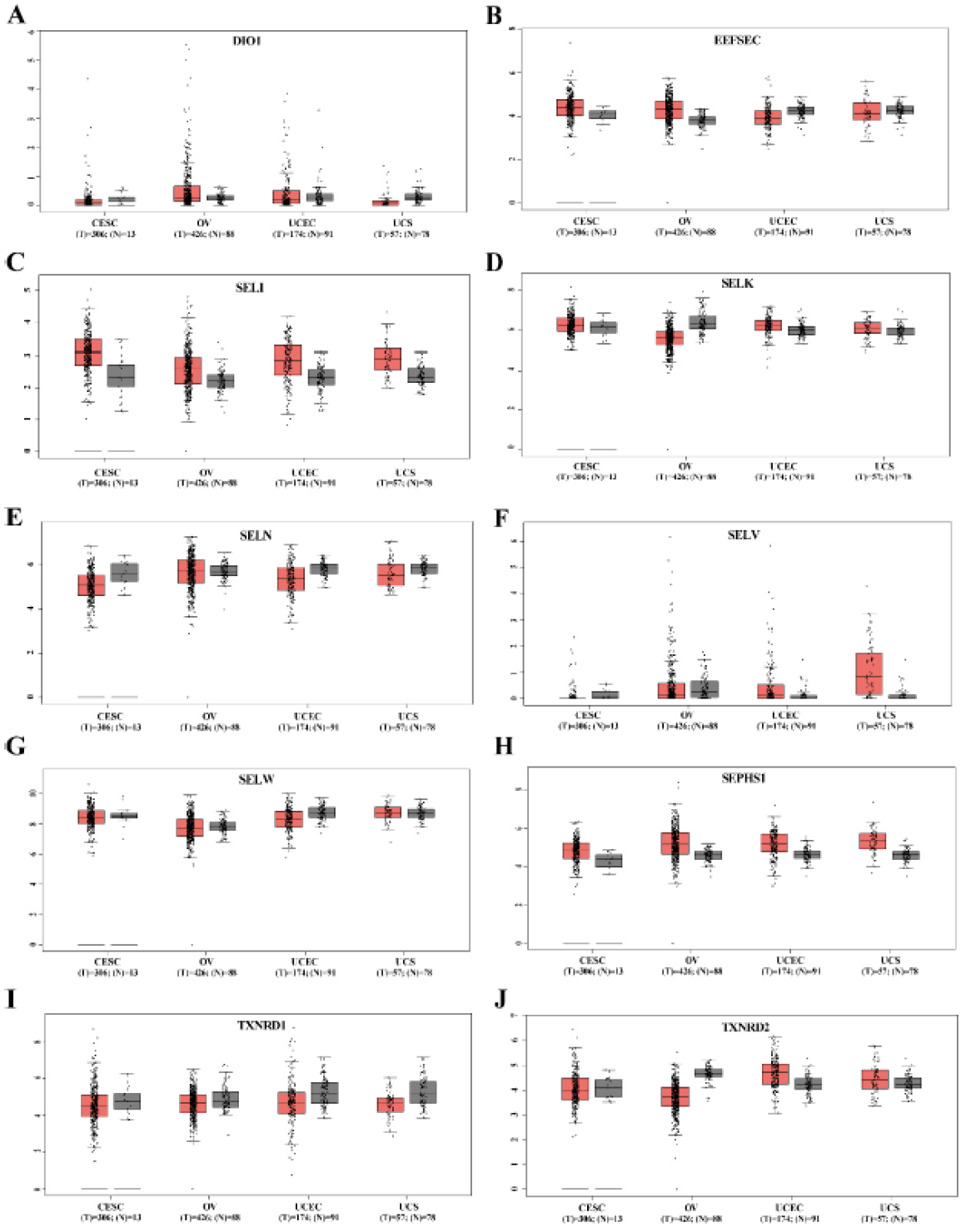
41. Zhao H, Tang J, Xu J, Cao L, Jia G, Long D, et al. Selenoprotein genes exhibit differential expression patterns between hepatoma HepG2 and normal hepatocyte LO2 cell lines. Biol Trace Elem Res. 2015;167:236-41.

42. Pothion H, Jehan C, Tostivint H, Cartier D, Bucharles C, Falluel-Morel A, et al. Selenoprotein T: essential oxidoreductase for endoplasmic reticulum homeostasis. Antioxid Redox Signal. 2020;33:1257-75.

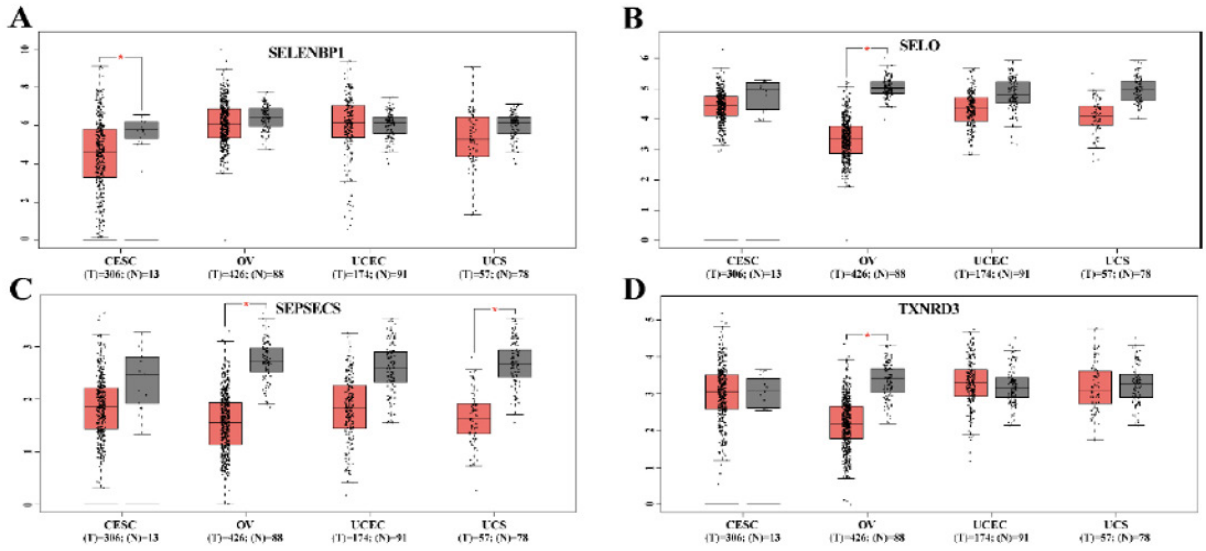
43. Zhuang W, Liu J, Li W. hsa-miR-33-5p as a therapeutic target promotes apoptosis of breast cancer cells *via* selenoprotein T. Front Med (Lausanne). 2021;8:651473.

44. Rocca C, Boukhzar L, Granieri MC, Alsharif I, Mazza R, Lefranc B, et al. A selenoprotein T-derived peptide protects the heart against ischemia/reperfusion injury by inhibiting apoptosis and oxidative stress. Acta Physiol (Oxf). 2018;223:e13067.

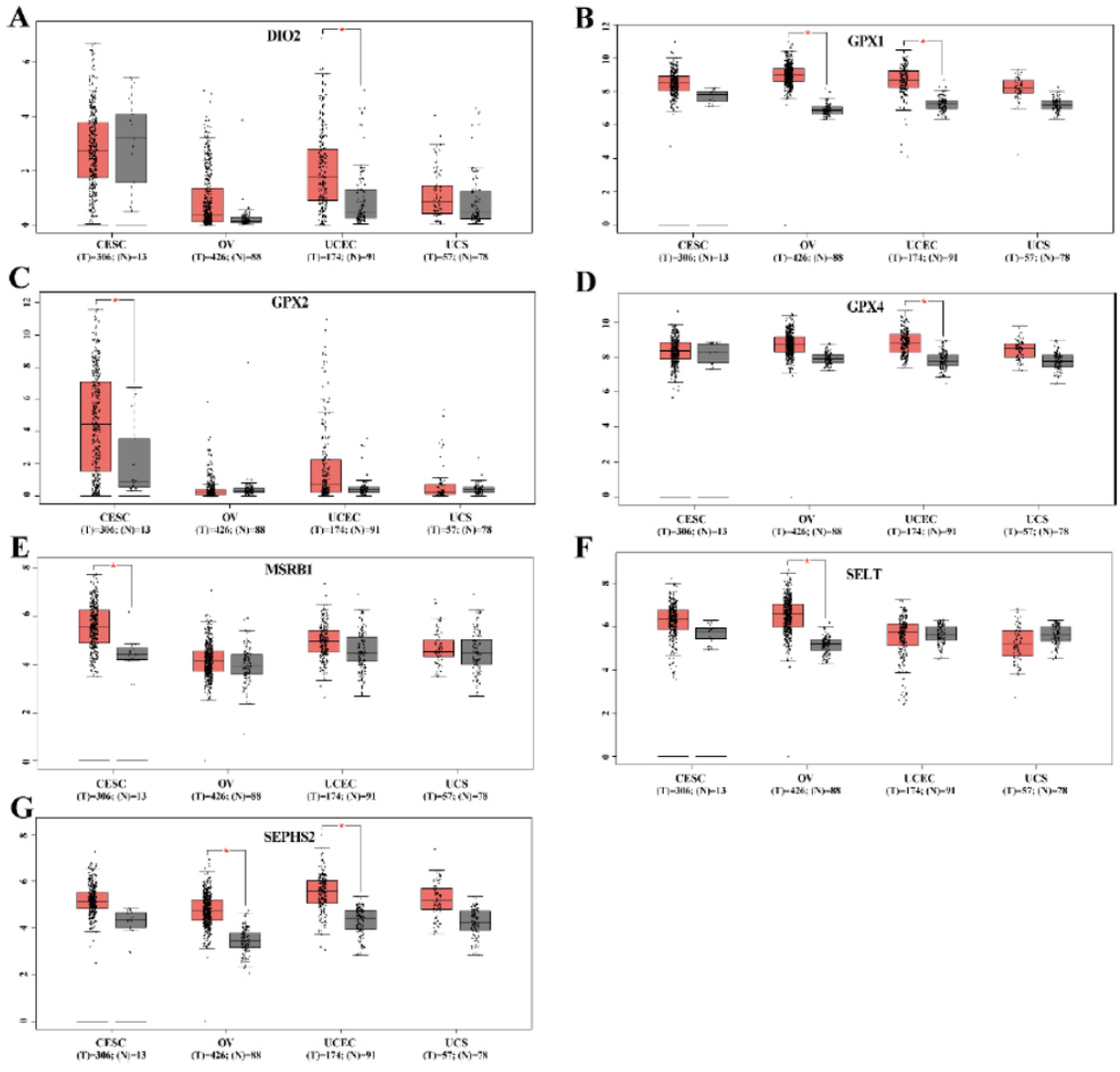
45. Vardhana SA, Hwee MA, Berisa M, Wells DK, Yost KE, King B, et al. Impaired mitochondrial oxidative phosphorylation limits T-cell self-renewal following exposure to persistent antigen. Nat Immunol. 2020;21:1022-33.



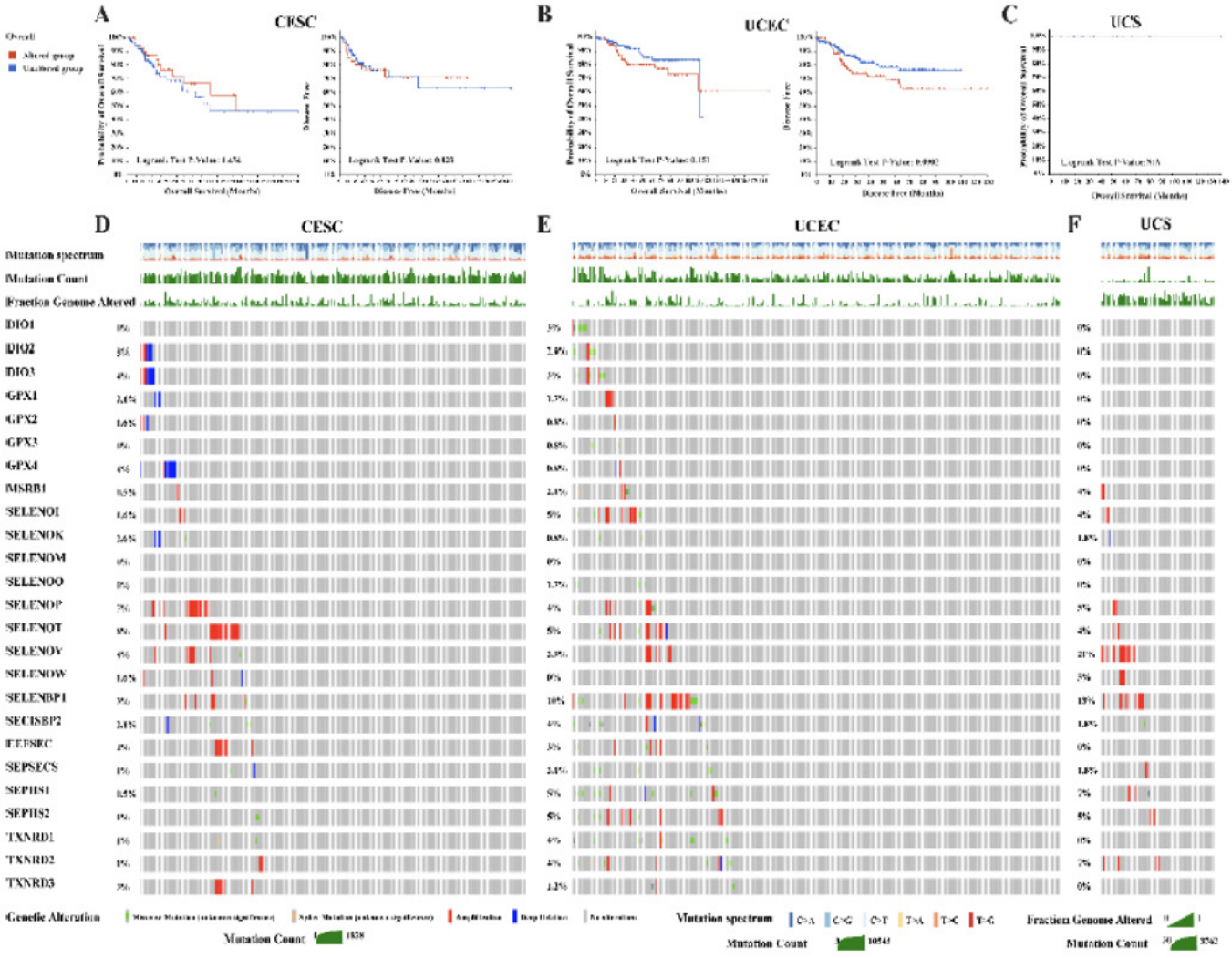
Supplementary Figure 1. mRNA expression of non-differential selenoprotein in patients with gynecological malignancies



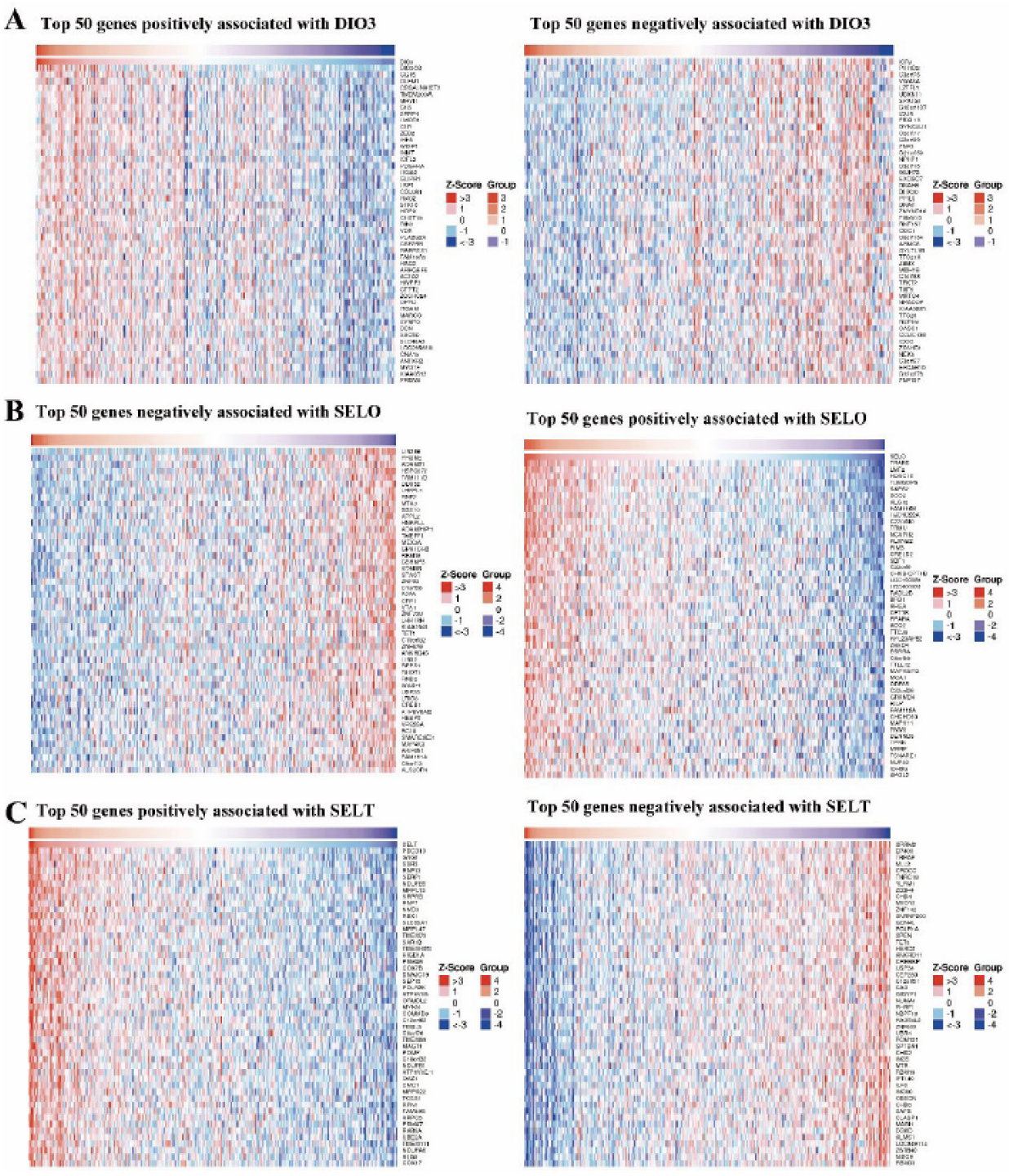
Supplementary Figure 2. Expression of downregulated selenoprotein in patients with gynecological malignancies



Supplementary Figure 3. Expression of upregulated selenoprotein in patients with gynecological malignancies

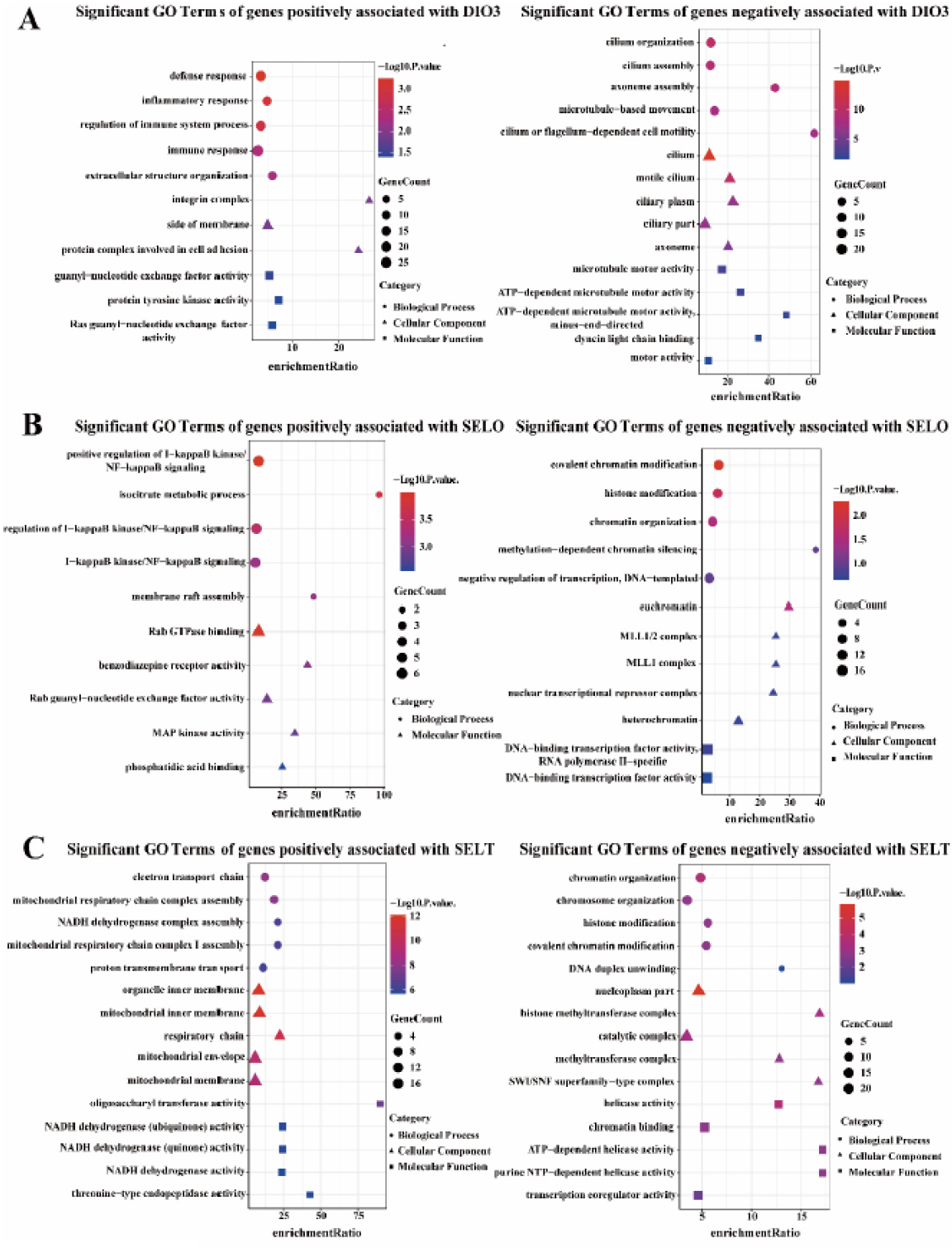


Supplementary Figure 4. Mutations and copy number variations of selenoprotein in cervical cancer, endometrial cancer and uterine carcinosarcoma (cBioPortal)



Supplementary Figure 5. Significant genes associated with DIO3, SELO, and SELT in ovarian cancer (LinkedOmics)

DIO3: Iodothyronine deiodinase 3, SELO: Selenoprotein O, SELT: Selenoprotein T



Supplementary Figure 6. Gene ontology enrichment analysis of genes associated with DIO3, SELO, and SELT in ovarian cancer (WebGestalt)

DIO3: Iodothyronine deiodinase 3, SELO: Selenoprotein O, SELT: Selenoprotein T

Supplementary Table 1. DIO3 in ovary cancer in Kaplan-Meier plotter

Clinicopathological feature	Overall survival (n=1657)			Progression- free survival (n=1436)		
	n	Hazard ratio	p	n	Hazard ratio	p
Histology						
Serous	1207	0.78 (0.67-0.92)	0.003	1104	1.26 (1.08-1.46)	0.003
Endometrioid	37	2.46 (0.41-14.75)	0.31	51	2.37 (0.93-6.03)	0.061
Stage						
1	74	0.27 (0.09-0.83)	0.015	96	2.03 (0.7-5.87)	0.18
1+2	135	0.31 (0.14-0.69)	0.0025	163	0.54 (0.3-0.96)	0.033
2	61	0.37 (0.12-1.14)	0.071	67	0.6 (0.3-1.18)	0.13
2+3	1105	0.76 (0.65-0.9)	0.0013	986	1.26 (1.08-1.46)	0.003
2+3+4	1281	0.77 (0.67-0.9)	0.00084	1148	1.29 (1.08-1.46)	7e-04
3	1044	0.75 (0.64-0.89)	0.00081	919	1.31 (1.11-1.49)	0.0011
3+4	1220	0.77 (0.66-0.89)	0.00059	1081	1.36 (1.16-1.58)	7.7e-05
4	176	1.47 (0.99-2.16)	0.052	162	1.75 (1.18-2.58)	0.0046
Grade						
1	56	0.39 (0.14-1.06)	0.055	37	0.43 (0.14-1.31)	0.13
1+2	380	0.77 (0.57-1.02)	0.071	293	1.3 (0.96-1.77)	0.092
2	324	0.8 (0.59-1.08)	0.15	256	1.47 (1.05-2.08)	0.026
2+3	1339	0.79 (0.68-0.92)	0.0019	1093	1.22 (1.05-1.42)	0.011
3	1015	0.78 (0.66-0.93)	0.0061	837	1.18 (0.99-1.39)	0.057
4	20	-	-	19	-	-
TP53 mutation						
Mutated	506	1.33 (1-1.78)	0.051	483	1.49 (1.19-1.86)	0.00041
Wild type	94	1.72 (0.91-3.23)	0.089	84	1.34 (0.79-2.26)	0.28
Average CA-125						
Below lower quartile	395	0.66 (0.5-0.87)	0.0025	326	0.61 (0.45-0.82)	0.0011
Debulk						
Optimal	801	0.61 (0.5-0.75)	1.7e-06	696	0.69 (0.57-0.84)	0.00014
Suboptimal	536	0.81 (0.64-1.01)	0.066	459	0.7 (0.56-0.88)	0.0023
Chemotherapy						
Platin	1409	0.84 (0.72-0.98)	0.025	1259	0.76 (0.66-0.88)	0.00012
Taxol	793	0.77 (0.64-0.93)	0.0064	715	1.2 (1-1.45)	0.053
Taxol+platin	776	0.76 (0.63-0.92)	0.0057	698	1.19 (0.99-1.44)	0.063
Avastin	50	0.25 (0.06-1.1)	0.048	50	0.63 (0.32-1.24)	0.18
Docetaxel	108	0.66 (0.37-1.16)	0.14	106	0.69 (0.42-1.13)	0.14
Gemcitabine	135	0.7 (0.44-1.12)	0.13	131	0.82 (0.55-1.2)	0.31
Paclitaxel	220	0.52(0.32-0.87)	0.011	229	1.17 (0.82-1.68)	0.38
Topotecan	119	1.52(0.99-2.31)	0.052	118	1.19 (0.81-1.77)	0.38

p<0.05 indicates statistical significance, CA-125: Cancer antigen-125

Supplementary Table 2. SELO in ovary cancer in Kaplan-Meier plotter

Clinicopathological feature	Overall survival (n=1657)			Progression- free survival (n=1436)		
	n	Hazard ratio	p	n	Hazard ratio	p
Histology						
Serous	523	0.8 (0.64-1)	0.054	483	0.77 (0.62-0.95)	0.016
Endometrioid	30	3.01 (0.42-21.42)	0.25	44	0.55 (0.17-1.77)	0.31
Stage						
1	51	0.27 (0.07-1.17)	0.064	74	0.26 (0.07-1)	0.036
1+2	83	0.6 (0.21-1.69)	0.33	115	2.01 (0.96-4.17)	0.057
2	32	4.82 (1.04-22.27)	0.028	41	3.02 (1.23-7.39)	0.011
2+3	458	0.83 (0.64-1.07)	0.15	465	0.84 (0.67-1.06)	0.14
2+3+4	519	0.86 (0.68-1.09)	0.22	535	0.87 (0.7-1.07)	0.17
3	426	0.81 (0.63-1.04)	0.1	424	0.85 (0.67-1.07)	0.16
3+4	487	0.85 (0.67-1.07)	0.17	494	0.87 (0.7-1.07)	0.19
4	61	1.41 (0.76-2.61)	0.27	70	0.61 (0.36-1.03)	0.064
Grade						
1	41	0.53 (0.18-1.56)	0.24	28	0.16 (0.04-0.62)	0.0026
1+2	203	0.7 (0.45-1.09)	0.12	189	0.57 (0.37-0.89)	0.011
2	162	0.81 (0.51-1.28)	0.31	161	0.69 (0.47-1.02)	0.063
2+3	554	0.79 (0.63-0.98)	0.034	476	0.79 (0.63-0.99)	0.04
3	392	0.7 (0.54-0.92)	0.01	315	0.71 (0.54-0.93)	0.011
4	18	0.32 (0.09-1.13)	0.063	19	-	-
TP53 mutation						
Mutated	124	1.29 (0.86-1.94)	0.21	124	1.51 (0.99-2.28)	0.053
Wild type	19	0.36 (0.11-1.21)	0.086	19	0.51 (0.19-1.38)	0.17
Average CA-125						
Below lower quartile	106	0.53 (0.32-0.89)	0.015	59	0.51 (0.28-0.93)	0.026
Debulk						
Optimal	243	0.63 (0.41-0.96)	0.032	240	0.7 (0.5-0.97)	0.031
Suboptimal	235	0.75 (0.56-1.01)	0.059	234	0.72 (0.55-0.95)	0.019
Chemotherapy						
Platin	478	0.86 (0.67-1.1)	0.22	502	0.86 (0.69-1.06)	0.16
Taxol	357	0.84 (0.61-1.16)	0.29	381	1.23 (0.96-1.59)	0.11
Taxol+platin	356	0.84 (0.62-1.16)	0.29	380	1.24 (0.96-1.59)	0.1
Avastin	-	-	-	-	-	-
Docetaxel	-	-	-	-	-	-
Gemcitabine	-	-	-	-	-	-
Paclitaxel	-	-	-	28	1.45 (0.62-3.4)	0.39
Topotecan	-	-	-	-	-	-

p<0.05 indicates statistical significance, CA-125: Cancer antigen-125

Supplementary Table 3. SELT in ovary cancer in Kaplan-Meier plotter

Clinicopathological feature	Overall survival (n=1657)			Progression- free survival (n=1436)		
	n	Hazard ratio	p	n	Hazard ratio	p
Histology						
Serous	1207	0.89 (0.76-1.04)	0.15	1104	1.26 (1.09-1.45)	0.002
Endometrioid	37	0.35 (0.06-2.09)	0.23	51	0.6 (0.2-1.82)	0.36
Stage						
1	74	1.96 (0.53-7.34)	0.31	96	0.37 (0.1-1.32)	0.11
1+2	135	0.4 (0.12-1.33)	0.12	163	0.59 (0.28-1.26)	0.17
2	61	0.17 (0.02-1.27)	0.048	67	0.44 (0.18-1.05)	0.058
2+3	1105	1.16 (0.98-1.36)	0.087	986	1.17 (1-1.36)	0.045
2+3+4	1281	0.92 (0.79-1.07)	0.27	1148	1.2 (1.04-1.39)	0.011
3	1044	1.19 (1-1.4)	0.047	919	1.2 (1.02-1.4)	0.025
3+4	1220	1.1 (0.94-1.28)	0.22	1081	1.24 (1.07-1.43)	0.0044
4	176	0.63 (0.43-0.92)	0.016	162	1.88 (1.17-3)	0.0075
Grade						
1	56	0.41 (0.13-1.26)	0.11	37	0.51 (0.17-1.52)	0.22
1+2	380	1.47 (1.06-2.02)	0.019	293	1.32 (0.94-1.84)	0.1
2	324	1.46 (1.03-2.06)	0.033	256	1.28 (0.92-1.8)	0.14
2+3	1339	0.84 (0.72-0.98)	0.024	1093	1.27 (1.06-1.43)	0.007
3	1015	0.78 (0.66-0.94)	0.0083	837	1.29 (1.08-1.54)	0.0047
4	20	-	-	19	-	-
TP53 mutation						
Mutated	506	0.87 (0.69-1.09)	0.23	483	1.36 (1.09-1.7)	0.007
Wild type	94	0.65 (0.33-1.29)	0.22	84	0.63 (0.35-1.14)	0.12
Average CA-125						
Below lower quartile	395	1.41 (1.06-1.88)	0.016	326	1.29 (0.98-1.7)	0.07
Debulk						
Optimal	801	1.17 (0.95-1.45)	0.14	696	1.31 (1.08-1.59)	0.0051
Suboptimal	536	0.72 (0.58-0.89)	0.0029	459	1.19 (0.96-1.48)	0.1
Chemotherapy						
Platin	1409	0.89 (0.77-1.03)	0.12	1259	1.2 (1.06-1.37)	0.0051
Taxol	793	0.87 (0.71-1.05)	0.15	715	1.18 (0.99-1.4)	0.061
Taxol+Platin	776	0.86 (0.7-1.04)	0.12	698	1.19 (0.99-1.41)	0.058
Avastin	50	0.53(0.18-1.61)	0.26	50	0.74 (0.37-1.47)	0.38
Docetaxel	108	0.41 (0.23-0.72)	0.0015	106	0.68 (0.38-1.22)	0.2
Gemcitabine	135	1.32 (0.87-2.02)	0.19	131	1.72 (1.14-2.6)	0.0086
Paclitaxel	220	0.65(0.41-1.05)	0.074	229	0.85 (0.6-1.18)	0.33
Topotecan	119	0.7 (0.47-1.04)	0.073	118	1.45 (0.98-2.14)	0.063

p<0.05 indicates statistical significance, CA-125: Cancer antigen-125

Supplementary Table 4. Correlation analysis between DIO3, SELO, SELT and relate genes and markers of immune cells in TIMER

Description	Gene markers	DIO3 none		Purity		SELO none		Purity		SELT none		Purity	
		Cor	p	Cor	p	Cor	p	Cor	p	Cor	p	Cor	p
T-cell	CD3D	0.292	****	0.079	0.22	0.065	0.26	0	0.99	0.261	****	0.169	**
	CD3E	0.312	****	0.102	0.11	0.147	*	0.098	0.11	0.21	***	0.107	0.09
	CD2	0.308	****	0.101	0.11	0.115	*	0.067	0.3	0.265	****	0.185	**
$CD8^+$ T-cell	CD8A	0.274	****	0.084	0.19	0.065	0.26	0.014	0.83	0.234	****	0.118	0.06
	CD8B	0.21	***	0.062	0.33	0.016	0.78	-0.045	0.48	0.299	****	0.211	***
$CD4^+$ T-cell	CD4	0.297	****	0.116	0.07	0.251	****	0.178	**	0.103	0.07	0.025	0.7
Th 1	TBX21	0.291	****	0.08	0.21	0.175	**	0.114	0.07	0.159	**	0.064	0.32
	STAT1	0.026	0.66	0.017	0.79	0.067	0.24	0.067	0.29	0.186	**	0.203	**
	STAT4	0.278	****	0.134	*	0.145	*	0.122	0.05	0.206	***	0.177	**
	IFNG	0.17	**	0.003	0.965	0.062	0.28	0.011	0.86	0.26	****	0.185	**
	TNF	0.214	***	0.099	0.12	0.236	****	0.26	****	0.186	**	0.098	0.12
Th 2	GATA3	0.315	****	0.204	**	0.116	*	0.027	0.67	0.134	*	0.077	0.23
	STAT6	0.08	0.16	0.085	0.18	0.108	0.06	0.045	0.48	-0.147	*	-0.092	0.15
	STAT5A	0.154	**	0.1	0.115	0.209	***	0.12	0.058	-0.089	0.12	-0.111	0.08
	IL13	0.145	*	0.197	**	0.065	0.261	0.08	0.208	0.01	0.856	0.025	0.691
Tfh	BCL6	0.146	*	0.226	***	0.286	****	0.259	****	-0.024	0.675	0.008	0.894
	IL21	0.017	0.762	0.013	0.837	0.066	0.256	0.118	0.062	0.183	**	0.134	*
Th 17	STAT3	0.252	****	0.179	**	0.208	***	0.16	*	0.073	0.205	0.062	0.332
	IL17A	0.12	*	0.08	0.206	0.053	0.36	0.022	0.728	0.126	*	0.107	0.093
Treg	FOXP3	0.267	****	0.101	0.11	0.164	**	0.09	0.156	0.207	***	0.143	*
	CCR8	0.215	***	0.106	0.095	0.028	0.626	-0.03	0.64	0.142	*	0.09	0.157
	STAT5B	0.173	**	0.143	*	0.172	**	-0.091	0.154	-0.13	*	-0.11	0.083
	TGFB1	0.333	****	0.126	*	0.201	***	0.081	0.204	0.191	***	0.159	*
T-cell exhaustion	PDCD1	0.25	****	0.116	0.067	0.134	*	0.071	0.261	0.222	****	0.161	*
	CTLA4	0.272	****	0.083	0.189	0.107	0.062	0.042	0.51	0.255	****	0.158	*
	LAG3	0.199	***	0.079	0.213	0.094	0.101	0.052	0.414	0.185	**	0.148	*
	HAVCR2	0.336	****	0.094	0.141	0.205	***	0.146	*	0.295	****	0.206	**
	GZMB	0.233	****	0.056	0.375	0.068	0.24	0.032	0.62	0.218	***	0.112	0.07
B-cell	CD19	0.046	0.426	-0.009	0.888	-0.015	0.801	-0.007	0.908	0.048	0.403	0.016	0.807
	CD79A	0.259	****	0.139	*	0.053	0.361	-0.003	0.962	0.097	0.091	-0.005	0.94
Monocyte	CD86	0.304	****	0.072	0.259	0.19	***	0.142	*	0.269	****	0.18	**
	CSF1R	0.342	****	0.117	0.066	0.295	****	0.23	***	0.117	*	0.003	0.96
TAM	CCL2	0.332	****	0.163	**	0.111	0.053	0.078	0.221	0.152	**	0.037	0.558
	CD68	0.35	****	0.13	*	0.204	***	0.146	*	0.261	****	0.185	**
	IL10	0.276	****	0.128	*	0.01	0.862	-0.046	0.472	0.205	***	0.108	0.09

Supplementary Table 4. continued

Description	Gene markers	DIO3 none		Purity		SELO none		Purity		SELT none		Purity	
M1 macrophage	NOS2	0.088	0.125	0.045	0.479	0.015	0.798	-0.036	0.571	0.057	0.319	0.072	0.258
	IRF5	0.148	**	0.071	0.264	0.268	****	0.242	***	0.136	*	0.132	*
	PTGS2	0.185	**	0.088	0.166	-0.039	0.498	-0.11	0.083	0.039	0.498	0.034	0.589
M2 macrophage	CD163	0.315	****	0.111	0.081	0.209	***	0.143	*	0.161	**	0.058	0.359
	VSIG4	0.297	****	0.066	0.299	0.129	*	0.071	0.267	0.221	***	0.097	0.129
	MS4A4A	0.3	****	0.078	0.218	0.115	*	0.045	0.476	0.26	****	0.167	**
Neutrophils	CEACAM	0.033	0.566	0.084	0.189	0.141	*	0.153	*	-0.141	*	-0.059	0.356
	ITGAM	0.378	****	0.168	**	0.281	****	0.2	**	0.149	**	0.081	0.202
	CCR7	0.283	****	0.121	0.057	0.137	*	0.051	0.421	0.148	**	0.086	0.174
Natural killer cell	KIR2DL1	0.128	*	0.079	0.216	0.049	0.393	0.033	0.603	0.074	0.244	0.066	0.253
	KIR2DL3	0.148	**	0.054	0.398	0.174	**	0.143	*	-0.002	0.978	-0.058	0.359
	KIR2DL4	0.202	***	0.042	0.507	0.12	*	0.061	0.337	0.122	*	0.044	0.485
	KIR3DL1	0.122	*	0.006	0.928	0.059	0.309	0.032	0.62	0.098	0.088	0.069	0.278
	KIR3DL2	0.164	**	0.063	0.324	0.089	0.124	0.041	0.522	0.079	0.172	0.051	0.42
	KIR3DL3	0.051	0.379	0.003	0.967	0.018	0.751	0.001	0.954	0.025	0.666	-0.006	0.929
	KIR2DS4	0.157	**	0.076	0.234	0.065	0.262	0.021	0.743	0.084	0.145	0.053	0.402
Dendritic cell	HLA-	0.281	****	0.056	0.376	0.221	***	0.2	**	0.186	**	0.051	0.427
	DPB1	-	-	-	-	-	-	-	-	-	-	-	-
	HLA-	0.18	**	0	0.996	0.096	0.096	0.054	0.4	0.137	*	0.021	0.741
	DQB1	-	-	-	-	-	-	-	-	-	-	-	-
	HLA-	0.254	****	0.085	0.181	0.136	*	0.118	0.062	0.241	****	0.116	0.066
	DRA												
	HLA-	0.266	****	0.062	0.328	0.173	**	0.141	*	0.193	***	0.067	0.29
	DPA1	-	-	-	-	-	-	-	-	-	-	-	-
	CD1C	0.333	****	0.157	*	0.127	*	0.067	0.295	0.061	0.293	-0.025	0.697
	NRP1	0.229	****	0.039	0.543	0.2	***	0.144	*	0.182	**	0.133	*
	ITGAX	0.36	****	0.166	**	0.355	****	0.327	****	0.16	**	0.08	0.207

*: p<0.05, **: p<0.01; ***: p<0.001, ****: p<0.000, Th: T helper cell, TAM: Tumor-associated macrophage

Supplementary Table 5. Correlation analysis between DIO3. SELO. SELT and relate genes and markers of immune cells in GEPIA

Description	Gene markers	DIO3				SELO				SELT			
		Tumor		Normal		Tumor		Normal		Tumor		Normal	
		Cor	p	Cor	p	Cor	p	Cor	P	Cor	p	Cor	p
T-cell	CD3D	0.25	****	-0.01	0.92	0.028	0.57	-0.12	0.28	0.22	****	0.04	0.74
	CD3E	0.29	****	0.06	0.56	0.15	**	-0.1	0.33	0.22	****	0.12	0.27
	CD2	0.31	****	0.06	0.58	0.12	*	-0.1	0.38	0.31	****	0.18	0.1
$CD8^+$ T-cell	CD8A	0.28	****	0.04	0.73	0.062	0.2	-0.11	0.33	0.29	****	0.19	0.08
	CD8B	0.21	****	-0.1	0.37	-0.03	0.54	-0.1	0.36	0.31	****	0.12	0.28
$CD4^+$ T-cell	CD4	0.34	****	0.34	**	0.25	****	0.003	0.97	0.28	****	0.32	**
Th 1	TBX21	0.34	****	-0.05	0.68	0.19	****	-0.07	0.53	0.27	****	-0.06	0.56
	STAT1	0.12	*	-0.11	0.31	0.2	****	-0.18	0.094	0.41	****	0.48	****
	STAT4	0.28	****	-0.33	**	0.19	****	0.15	0.17	0.28	****	-0.03	0.78
	IFNG	0.17	***	-0.33	**	0.058	0.23	-0.03	0.76	0.26	****	-0.16	0.13
	TNF	0.24	****	-0.05	0.65	0.24	****	-0.12	0.25	0.29	****	0.1	0.34
Th 2	GATA3	0.28	****	-0.04	0.73	0.097	*	-0.11	0.31	0.14	**	-0.04	0.71
	STAT6	0.13	**	-0.26	*	0.33	****	0.42	****	0.14	**	-0.26	*
	STAT5A	0.24	****	0.43	****	0.3	****	-0.05	0.67	0.23	****	0.27	*
	IL13	0.12	*	0.07	0.55	0.17	***	0.03	0.77	-0.016	0.74	-0.09	0.4
Tfh	BCL6	0.23	****	0.05	0.63	0.39	****	-0.06	0.56	0.22	****	-0.01	0.91
	IL21	0.1	*	0.18	0.087	0.015	0.75	0.05	0.63	0.14	**	0.11	0.3
Th17	STAT3	0.31	****	0.27	**	0.31	****	-0.16	0.13	0.39	****	0.3	**
	IL17A	0.027	0.58	-0.13	0.21	0.027	0.58	-0.14	0.19	0.065	0.18	0.25	*
Treg	FOXP3	0.28	****	-0.07	0.5	0.2	****	0.02	0.89	0.3	****	0.05	0.63
	CCR8	0.23	****	-0.06	0.61	0.12	0.017	-0.2	0.056	0.31	****	0.1	0.34
	STAT5B	0.24	****	0.18	0.098	0.28	****	0.23	*	0.25	****	0.19	0.08
	TGFB1	0.37	****	0.12	0.25	0.27	****	-0.18	0.1	0.38	****	0.28	**
T-cell exhaustion	PDCD1	0.26	****	0.2	0.067	0.17	***	-0.09	0.4	0.28	****	0.22	*
	CTLA4	0.28	****	-0.06	0.58	0.13	**	-0.26	*	0.28	****	0.2	0.06
	LAG3	0.2	****	0.17	0.1	0.13	**	0.34	**	0.18	***	-0.15	0.15
	HAVCR2	0.36	****	0.34	**	0.22	****	-0.29	**	0.43	****	0.47	****
	GZMB	0.22	****	0.05	0.63	0.12	*	0.03	0.78	0.23	****	-0.01	0.94
B-cell	CD19	0.085	0.078	-0.37	***	0.07	0.15	0.26	*	0.095	*	-0.24	*
	CD79A	0.22	****	0.14	0.21	0.028	0.56	-0.26	*	0.11	*	0.08	0.45
Monocyte	CD86	0.35	****	0.25	*	0.18	***	-0.38	***	0.4	****	0.46	****
	CSF1R	0.37	****	0.25	*	0.31	****	-0.16	0.13	0.32	****	0.45	****
TAM	CCL2	0.34	****	-0.06	0.58	0.13	0.008	-0.23	*	0.25	****	0.25	*
	CD68	0.38	****	0.14	0.21	0.24	****	-0.44	****	0.43	****	0.56	****
	IL10	0.32	****	0.24	*	0.11	*	-0.23	*	0.41	****	0.37	***

Supplementary Table 5. continued

Description	Gene markers	DIO3				SELO				SELT			
		Tumor		Normal		Tumor		Normal		Tumor		Normal	
M1 Macrophage	NOS2	0.18	***	-0.19	0.075	0.16	***	0.13	0.23	0.22	****	-0.01	0.95
	IRF5	0.2	****	0.21	*	0.34	****	-0.12	0.28	0.34	****	0.38	****
	PTGS2	0.26	****	-0.06	0.6	0.0082	0.87	-0.1	0.33	0.22	****	-0.06	0.57
M2 Macrophage	CD163	0.29	****	0.52	****	0.18	***	-0.32	**	0.28	****	0.46	****
	VSIG4	0.35	****	0.46	****	0.15	**	-0.27	*	0.36	****	0.5	****
	MS4A4A	0.34	****	0.36	***	0.14	**	-0.29	**	0.4	****	0.43	****
Neutrophils	CEACAM8	0.09	0.064	-0.15	0.16	0.14	**	-0.04	0.69	-0.034	0.48	-0.07	0.53
	ITGAM	0.41	****	0.32	**	0.31	****	-0.25	*	0.36	****	0.44	****
	CCR7	0.28	****	0.21	*	0.2	****	-0.16	0.14	0.29	****	0.19	0.07
Natural killer cell	KIR2DL1	0.18	***	0.13	0.23	0.14	**	-0.05	0.62	0.19	****	-0.03	0.77
	KIR2DL3	0.25	****	0.01	0.95	0.17	****	-0.16	0.15	0.2	****	0.02	0.82
	KIR2DL4	0.24	****	0.05	0.65	0.19	****	-0.15	0.17	0.23	****	0.1	0.37
	KIR3DL1	0.19	****	0.05	0.65	0.11	*	0.03	0.81	0.18	***	0.01	0.93
	KIR3DL2	0.21	****	0.05	0.68	0.21	****	-0.05	0.66	0.19	****	-0.02	0.85
	KIR3DL3	0.09	0.064	0.14	0.19	0.12	*	0	1	0.069	0.16	0.08	0.44
	KIR2DS4	0.13	**	0.1	0.36	0.14	**	0.07	0.49	0.2	****	-0.11	0.33
Dendritic cell	HLA-DPB1	0.33	****	-0.13	0.24	0.19	****	-0.25	*	0.23	****	0.17	0.12
	HLA-DQB1	0.16	**	-0.15	0.16	0.06	0.21	-0.12	0.26	0.14	**	0.1	0.34
	HLA-DRA	0.3	****	0.01	0.9	0.13	**	-0.32	**	0.29	****	0.35	****
	HLA-DPA1	0.32	****	-0.01	0.92	0.16	***	-0.21	*	0.27	****	0.27	*
	CD1C	0.32	****	0.15	0.16	0.12	*	0.002	0.98	0.15	**	0.19	0.08
	NRP1	0.27	****	-0.15	0.15	0.23	****	-0.23	*	0.34	****	0.43	****
	ITGAX	0.35	****	-0.1	0.36	0.39	****	-0.05	0.63	0.31	****	-0.02	0.88

*: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001, Th: T helper cell, TAM: Tumor-associated macrophage