Expression, Prognosis, and Regulation of ULBP1, ULBP2, and ULBP3 in Human Breast Cancer

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Research

Keywords: breast cancer, NKG2D, NKG2D ligands, prognosis, immune cell

Posted Date: June 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-574086/v1

Expression, Prognosis, and Regulation of ULBP1, ULBP2, and ULBP3 in Human Breast Cancer

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Funding information

The stable support program project of higher institutions of Shenzhen Science and Technology Innovation Committee, research on the mechanism of cancer secreted lama4 in promoting immune escape of pancreatic cancer cells through regulating Treg cells and related immunotherapy, 2021 / 01-2022 / 12 [this year's topic has not been signed a contract, but there are public documents]

Abstract

The expression of NKG2D ligands (NKG2DLs), induced by stress or malignant transformation, is considered to mark dysfunctional cells for elimination by NK cells or cytotoxic lymphocytes via NKG2D/NKG2DLs pathway. ULBP1, ULBP2, and ULBP3 (ULBP1-3), three members of NKG2DLs, are common expressed in breast cancer. We analyzed the expression of ULBP1-3 in breast cancer and healthy control tissues with several databases, and found that breast cancer had a higher mRNA level of ULBP1 and ULBP2, and a higher protein level of ULBP1-3. Analysis with the bc-GenExMiner database showed that the expression of ULBP1-3 were down-regulated by the wild type P53, PR, and HER2+ in breast cancer. Except for ULBP1, ULBP2 and ULBP3 were associated with poor prognosis in breast cancer. The analysis of the correlated genes suggested that ULBP1-3 have some common pathways including NK cell-mediated cytotoxicity, microRNA in cancer, and IL-17 signaling pathway, whereas they also have their own pathways. But ULBP1-3 expression were also a significantly negative correlation with few immune markers on NK and central memory CD8+ T. The correlations and co-occurrence between ULBP1-3 and the other ligands by using TIMER and cbioportal databases showed the same form proteins or the proteins in the same family were more likely to change at the same time. Together with all these findings, increased ULBP2 and ULBP3 were correlated with poor prognosis and various markers on immune cells. These conclusions indicated that ULBP2 and ULBP3 could serve as potential biomarkers to assess prognosis and they were strong correlation with various markers in immune cells.

KEYWORDS

breast cancer, NKG2D, NKG2D ligands, prognosis, immune cell

1 | INTRODUCTION

Breast cancer is a leading cause of cancer-related death worldwide in women. Nowadays, the incidence and mortality of breast cancer is increasing in China (Jemal et al., 2007). To date, current primary therapies including surgical resection, chemotherapy, and radiotherapy demonstrate high complete response rates (Bajgain et al., 2018). However, due to the high rate of relapse, the five-year survival rate for advanced breast cancer is still poor, highlighting the need for effective therapeutic strategies. As an alternative to traditional treatments, immunotherapeutic approaches including vaccination, checkpoint inhibitors, monoclonal antibodies (mAbs), adoptive cellular immunotherapy may be able to specifically target tumor cells. However, the study of target antigens which are expressed broadly in breast cancer is required.

Natural killer group 2 member D (NKG2D), a C-type lectin-like receptor, controls the activation of NK cell and cytotoxic T lymphocyte. It is expressed on all natural killer (NK) cells, CD8+ T cells, NKT cells, a small subset of γδ T cells, and CD4+ T cells. NKG2D binds to various stress-induced ligand molecules and then facilitates the activation of immune cells leading to the production of numerous cytokines and target cell elimination. These biological functionss have therapeutic potential as NKG2D ligands (NKG2DLs) are frequently presented on the various tumor from multiple origins, whereas their expression in healthy cells is restricted. In humans, NKG2DLs are classified into two categories: MICA/B and ULBP1-6. Recently, a series of experiments have demonstrated that one or more NKG2DLs are expressed on the surface of all cell types in a wide variety of tumors (Spear, Wu, Sentman, & Sentman, 2013). The efficiency of NKG2D-mediated lysis by cytotoxic immune cells has been shown to related to the surface density of NKG2DLs on target cells. Absent or low expression of NKG2DLs in patients contributes to immune system evasion (Baragaño Raneros et al., 2015; Paczulla et al., 2019). However, the overexpression of NKG2DLs induced by the chemotherapy treatments may increase levels of soluble NKG2DLs in pancreatic cancer (Kohga et al., 2009; Morisaki et al., 2011). These soluble molecules impaired immune cell activity by reducing the surface

expression of NKG2D in immune cells (Groh, Wu, Yee, & Spies, 2002; Song, Kim, Cosman, & Choi, 2006). Therefore, it is important to understand the underlying mechanism of NKG2DLs expression and function in cancer.

ULBP1-3, expressed in glycophosphatidylinositol (GPI)-anchored form, are found expressed in 90-100% of breast tumor (de Kruijf et al., 2012; Ohashi, Eagle, & Trowsdale, 2010). Despite recent progress, the diverse expression patterns, prognostic values, and function of ULBP1-3 in breast cancer have been insufficiently studied. In the present study, we used the public databases to explore the expression level and prognosis value of ULBP1-3 in patients with breast cancer. Next, we investigated the correlations of NKG2DLs with tumor-infiltrating immune cells and their task in tumor immunity. Finally, we evaluate the pathway related to ULBP1-3 as well as the relationship between ULBP1-3 and other NKG2DLs. This study revealed the potential role of ULBP1-3 in breast cancer and provides a new understanding of the pathogenesis and treatment of breast cancer.

2 | MATERIALS AND METHODS

2.1 | *ULBP1-3 expression analysis*

The mRNA levels of ULBP1-3 in breast cancer and normal tissue were analyzed by using the Oncomine database (https://www.oncomine.org/resource/login.html) and Tumor Immune Estimation Resource (TIMER) database (https://cistrome.shinyapps.io/timer/) (T. Li et al., 2017; Rhodes et al., 2007). In the Oncomine database, the cut-off of fold change (FC) and p value were defined as 1.5 and 0.05, respectively. To validate the protein expression of ULBP1-3, Human Protein Atlas (HPA) database (http://www.proteinatlas.org/) was used (Thul & Lindskog, 2018).

2.2 | *Correlation analysis of NKG2DLs Expression and different Clinicopathological Parameters*

The Breast Cancer gene-expression miner v4.5 (http://bcgenex.centregauducheau.fr/BC-GEM/GEM-Accueil.php?js=1), an online

web tool, was employed for analyzing the relationship between ULBP1-3 expression and clinicopathological parameters of breast cancer patients. The clinicopathological features including age, HER2 receptor status (HER2) (by IHC), estrogen receptor status (ER) (by immunohistochemistry [IHC]), P53 status, progesterone receptor status (PR) (by IHC), triple-negative BC status, and nodal status (N) were selected for further analysis.

2.3 | *Prognostic analysis of ULBP1-3 in breast cancers*

Kaplan-Meier Plotter (http://kmplot.com/analysis/) was used to evaluate the potential prognostic of ULBP1-3 (Lánczky et al., 2016). The survival curves including overall survival (OS), post-progression survival (PPS), and distant metastasis-free survival (DMFS) was generated and hazard ratio (HR) with 95% confidence intervals (CI) as well as the log-rank P-value were calculated.

2.4 | *Immune infiltration and immune marker analysis related to ULBP1-3 expression*

To estimate the correlation of ULBP1-3 expression with diverse tumor-infiltrating immune cells and gene markers on various immune cells, TIMER database was used. The immune cells include macrophages, B cells, dendritic cells, neutrophils, CD4+ T cells, CD8+ T cells as well as tumor purity. Further, the association of the gene markers on various immune cells were analyzed. The gene markers were expressed in NK cell, NKT cell, γδ T cells, activated CD8 T cell, activated CD4 T cell, central memory CD8+ T cell, central memory CD4+ T cell, effector memory CD8+ T cell, effector memory CD4+ T cell, NKG2D pathway, and T cell exhaustion. The gene markers were mainly referenced in prior studies (Ru et al., 2019). The threshold for the significant correlation was determined according to the following values: $|cor|>0.3$, p-value <0.05.

2.5 | *The correlation between ULBP1-3 and other different expression of gene analysis*

We analyzed the association between ULBP1-3 expression and mRNA in breast cancer using LinkedOmics database (http://www.linkedomics.org/) (Vasaikar, Straub, Wang, & Zhang, 2018). Pearson's correlation coefficient was applied and all results were presented in volcano plots, heat maps, or scatter plots in the LinkFinder module. Then the results from the LinkFinder were used to enrich the KEGG pathway in the LinkInterpreter module. We selected GSEA and then the "clusterProfiler" package in R in the LinkInterpreter module.

2.6 | *The relationship of ULBP1-3 and other NKG2DLs analysis*

ULBP1-3 correlated with other ligands were obtained using bc-GenExMiner v4.5. While the other ligands that co-occurred with ULBP1-3 were determined by using the cBio Cancer Genomics Portal (cbioportal, http://cbioportal.org) database.

3 | RESULTS

3.1 | *NKG2DLs Expression in breast cancer*

In the Oncomine databases, ULBP1, ULBP2, and ULBP3 were lower in normal tissues (Figure 1A). While higher expression of ULBP1 and ULBP3 were also observed in breast cancer compared with that in normal tissues in the TIMER database. However, overexpression of ULBP3 was found in healthy controls (Figure 1B).

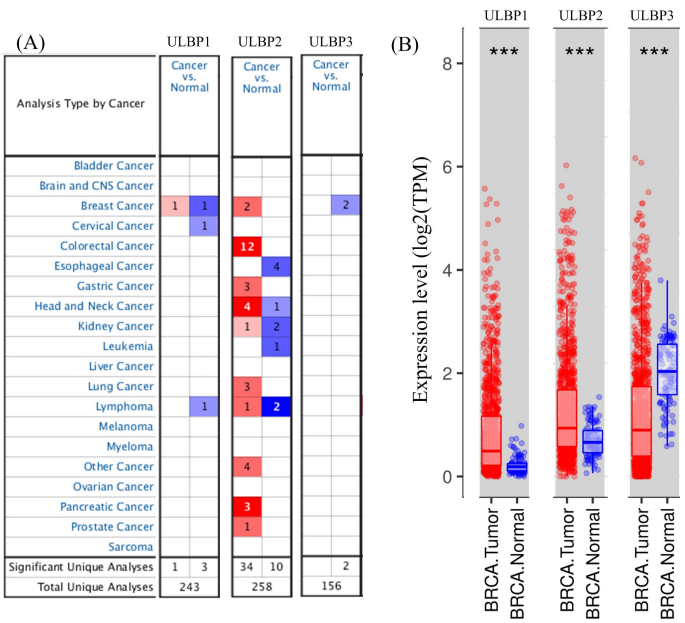


Figure 1 ULBP1, ULBP2, and ULBP3 expression in breast cancer and normal tissues. (A), NKG2DLs expression in the Oncomine database. (B), ULBP1, ULBP2, and ULBP3 expression expression in the TIMER databas

The Human Protein Atlas database was used to analyzed the protein expression of ULBP1, ULBP2, and ULBP3 by immunohistochemistry (IHC) in breast cancer tissues and the related normal tissues. ULBP1, ULBP2, and ULBP3 were almost negative staining in normal tissue. Compared with normal breast tissues, the protein of ULBP1, ULBP2, and ULBP3 were more highly expressed in the breast cancer tissues (Figure 2). It is worth noting that the compared result of ULBP3 protein expression in breast cancer and healthy tissues is contrary to that of ULBP3 mRNA expression. It may be that translation of ULBP3 is restricted in normal tissues.

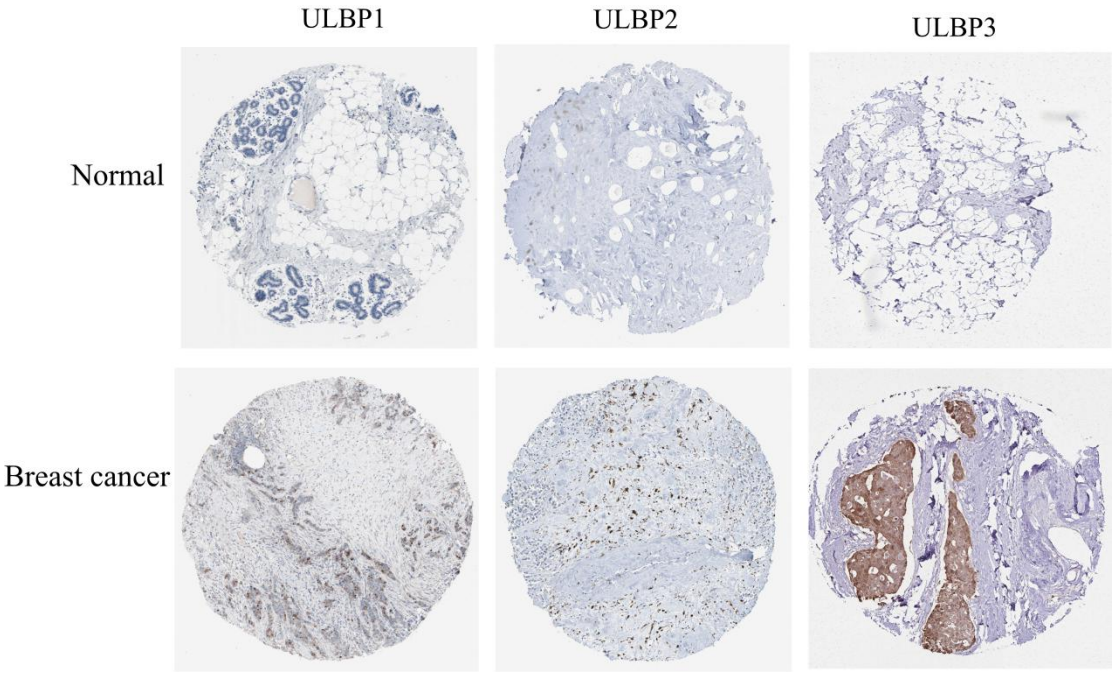


Figure 2 IHC images of ULBP1, ULBP2, and ULBP3 detected in the HPA

3.2 | *Correlation between NKG2DLs Expression and different Clinicopathological Parameters of breast cancer patients.*

According to different clinicopathological parameters, We compared the mRNA expression of ULBP1, ULBP2, and ULBP3 between groups of patients by Welch's test in bc-GenExMiner database. As for age, mRNA levels of ULBP1, ULBP2, and

ULBP3 were down-expression in ≥51 y patients group. For the nodal status criterion, there was no significant difference between positive and negative nodal status. The expression level of ULBP1, ULBP2, and ULBP3 was negatively controlled by estrogen receptor (ER), progesterone receptor (PR), HER2 receptor (HER2), and wild type P53. Comparing PR and ER status, the negative effect of ER was higher than that of PR for ULBP1-3 mRNA expression level (Supplemental Figure 1). Furthermore, they were up-expression in triple-negative breast cancer (TNBC) groups, compared with non-TNBC groups.

3.3 | *Investigate the clinical significance of ULBP1-3 in breast cancer patients*

Many studies have shown that the prognostic of tumor patients affect by NKG2DLs. To study the correlation between NKG2DLs mRNA expression and clinical features, we evaluated the prognostic value of NKG2DLs in patients with breast cancer by using Kaplan-Meier Plotter tools. As shown in Figure 3, ULBP1 expression did no effect on OS (p=0.15), PPS (p=0.15), (DMFS, p=0.13). ULBP2 over-expression was associated with poor OS (p=4.7e-05), PPS (p=0.00049), and DMFS (p=0.00074). While ULBP3 over-expression was only associated with poor OS (p=0.0025).

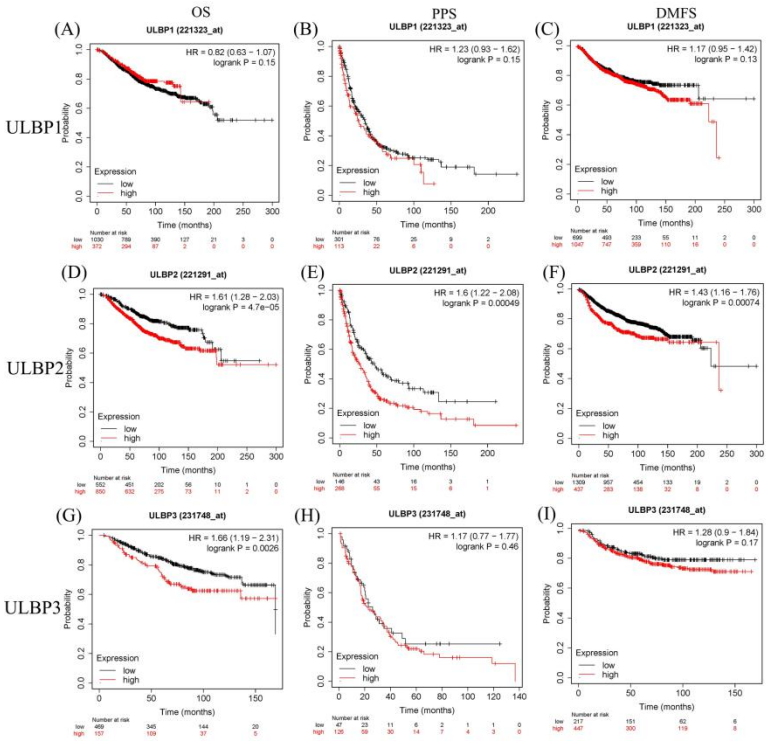


Figure 3 Clinical significance of ULBP1-3 in breast cancer patients. (A-C), Survival curves of OS, PPS, and DMFS for ULBP1 in breast cancer, respectively; (D-F), Survival curves of OS, PPS, and DMFS for ULBP2 in breast cancer, respectively; (G-I), Survival curves of OS, PPS, and DMFS for ULBP2 in breast cancer, respectively

3.4 | *Analysis of the ULBP1-3 functional network and pivotal role in breast cancer*

To broaden the understanding of ULBP1-3 biological role with the other correlated genes in breast cancer, the correlation genes of ULBP1-3 were analyzed by using the function module of LinkedOmics from 1093 patients with LIHC in the TCGA dataset. There were 2284, 2114, and 2947 genes significantly correlated with ULBP1, ULBP2, and ULBP3, respectively (Supplemental table 1-3). KEGG enriched by the significant correlation genes of ULBP1-3 were conducted by GSEA in LinkedOmics. This results showed that ULBP1-3 not only having a similar functioning but also have their own unique function (Figure 4). The KEGG pathway including NK cell mediated cytotoxicity, microRNA in cancer, IL-17 signaling pathway, ribosome biogenesis in eukaryotes, glycosphingolipid biosynthesis, bacterial invasion of epithelial were shared by ULBP1-3. The unique pathways to ULBP1 were RNA transport, DNA replication, cellular senescence, aminoacyl-tRNA biosynthesis, and Epstein-Barr virus infection pathways. The significant correlation genes enriched in ULBP2 were osteoclast differentiation, rheumatoid arthritis, regulation of actin cytoskeleton. We also identified four specific pathways including cytokine-cytokine receptor interaction, ferroptosis, adherens junction, focal adhesion, and hippo signaling pathway enriched by the significant correlation genes of ULBP3. Thus, a widespread impact of ULBP1-3 on the global transcriptome.

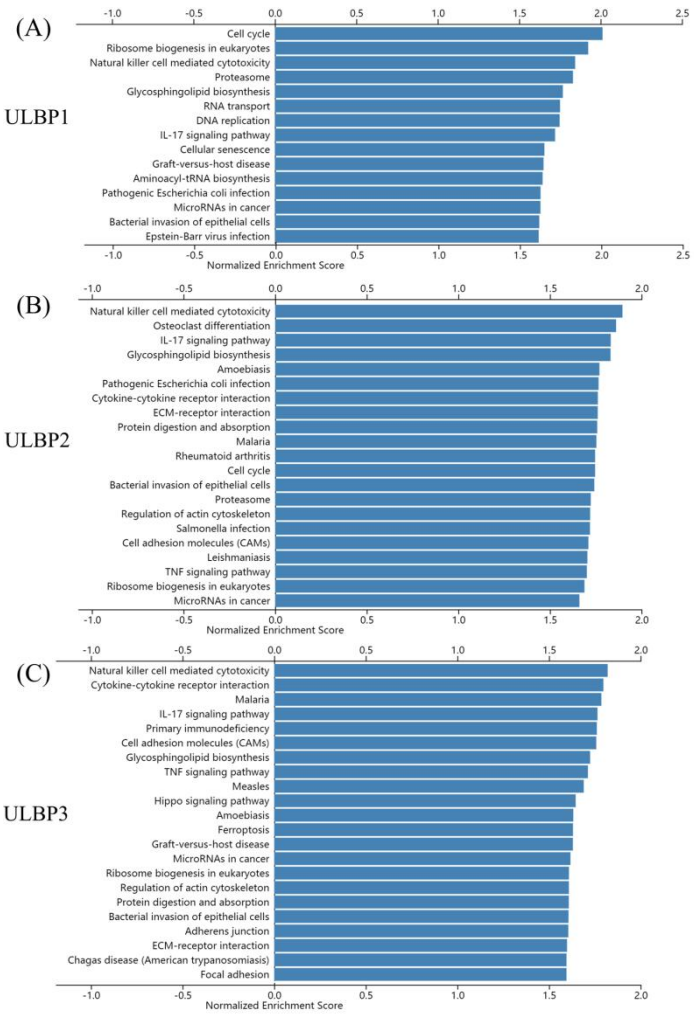


Figure 4 Significantly enriched KEGG pathways of ULBP1-3 in breast cancer patients (FDR<0.05). (A), KEGG pathways of ULBP1; (B), KEGG pathways of ULBP2; (C), KEGG pathways of ULBP3

3.5 | *ULBP1-3 expression is associated with the level of immune infiltration in breast cancer*

In all subtypes of breast cancer, Tumor-infiltrating lymphocytes (TILs) play an important role in improving clinical outcomes (Stanton & Disis, 2016). We tried to investigate whether ULBP1-3 associate with TILs in breast cancer by using the TIMER database. We found there were no high correlations between ULBP1-3 expression and immune cell infiltration levels. ULBP1-3 expression had weak correlations (|cor|≤0.3) with the majority of immune cell infiltration levels and tumor purity (Figure 5). These results indicated that ULBP1-3 could not modulate immune

infiltrating cells into breast cancer tissues and their expression may be from cells in the tumor microenvironment.

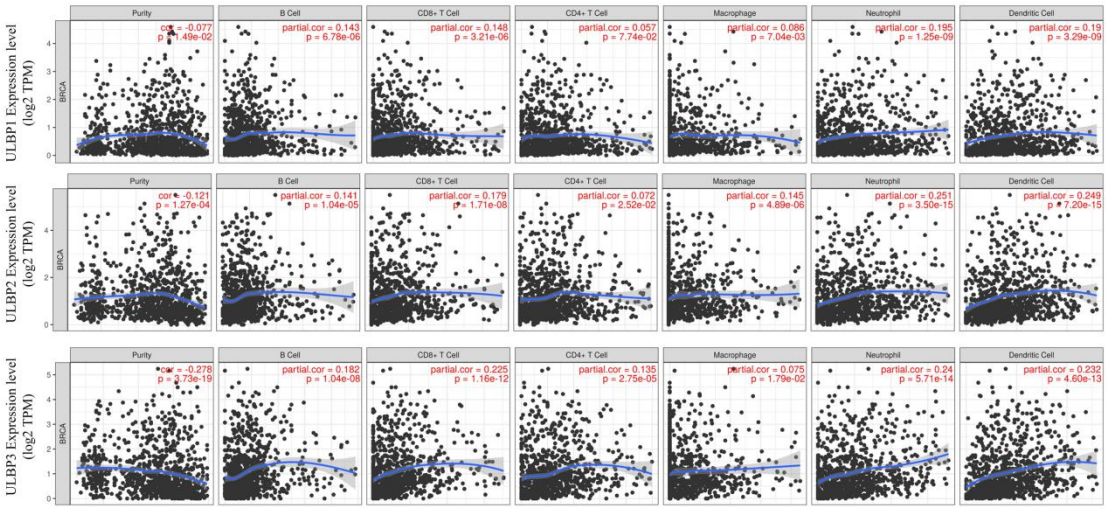


Figure 5 Correlation of NKG2DLs expression with immune infiltration level in breast cancer cohort

3.6 | *Correlation Analysis Between ULBP1-3 Expression and Immune signatures*

Varying expression levels of ULBP1-3 in breast cancer patients may be associated with functional differences in immune regulation. To investigate the correlation between ULBP1-3 and immune functions in breast cancer, the correlations between the expression of ULBP1-3 and the immune marker of different immune cells were studied in the TIMER database. We initially evaluated the immune cells, which express the receptor of ULBP1-3, including NK cell, activated CD8+ T cells, NK T cells, activated CD4+ T cells, and γδ T cells. The results revealed that ULBP1-3 expression level was significantly correlated with most immune marker sets of activated CD4+ T cells, NK T cells, and γδ T cells in breast cancer (Figure 6A, C, E). More remarkable, ULBP1-3 were not only significantly positive correlations with the marker of NK cells but also were significantly negative correlations with the marker of NK cells (Figure 6D). The negatively correlated genes mainly include ERBB3, BCL2, GLS2, and FUCA1 in NK cells. As for activated CD8+ T cells, only the

expression of MPZL1 was significantly positive correlations with the expression of ULBP1-3 (Figure 6B).

Expression of NKG2DLs in some mouse tumor models strongly enhances the induction of anti-tumor memory responses (Diefenbach, Jensen, Jamieson, & Raulet, 2001). In this study, results showed the expression levels of ULBP1-3 have positive correlations with various markers expression in effector memory CD8+ T, central memory CD4+ T, and effector memory CD4+ T (Figure 6F-I). Among the four memory T cells, the number of significantly and positively correlated genes in central memory CD4+ T cell was more than that in effector memory CD8+ T, central memory CD8+ T, and effector memory CD4+ T, and indicating central memory CD4+ T cell paly an important role for anti-tumor memory responses. For central memory CD8+ T cell, only TNFRSF11A was significantly and positively correlated with the expression of ULBP3, and RARA was significantly and negatively correlated with the expression of ULBP1-3 (Figure 6F). In effector memory CD4+ T, except for EZH2 significant correlation with ULBP1, the rest of significantly and positively correlated genes were ULBP3 with IL34, DARS, WIP1, CD300E, REPS1 (Figure 6G). What is more, RARA is the only significantly and negatively correlated marker in the memory cells (Figure 6I). All these results thus suggest that ULBP1-3 play an important role in central memory CD4+ T and effector memory CD8+ T, whereas the expression level of ULBP3 plays an important role in central memory CD8+ T.

Upon binding to ligands, the activating NKG2D receptor can specifically interact with DAP10 and DAP12 mediating cytotoxic responses, secretion of cytokines, and chemokines (Diefenbach et al., 2001; Jelenčić, Lenartić, Wensveen, & Polić, 2017; Stojanovic, Correia, & Cerwenka, 2018). However, the gene expression level of NKG2D, DAP10, IFN-α, and IFN-γ have not significantly positive correlations with ULBP1-3 expression (Figure 6J). In the markers related to T cell exhaustion, results showed that only the expression levels of GZMB and CTLA4 were significantly positive correlations with ULBP3 expression (Figure 6K).

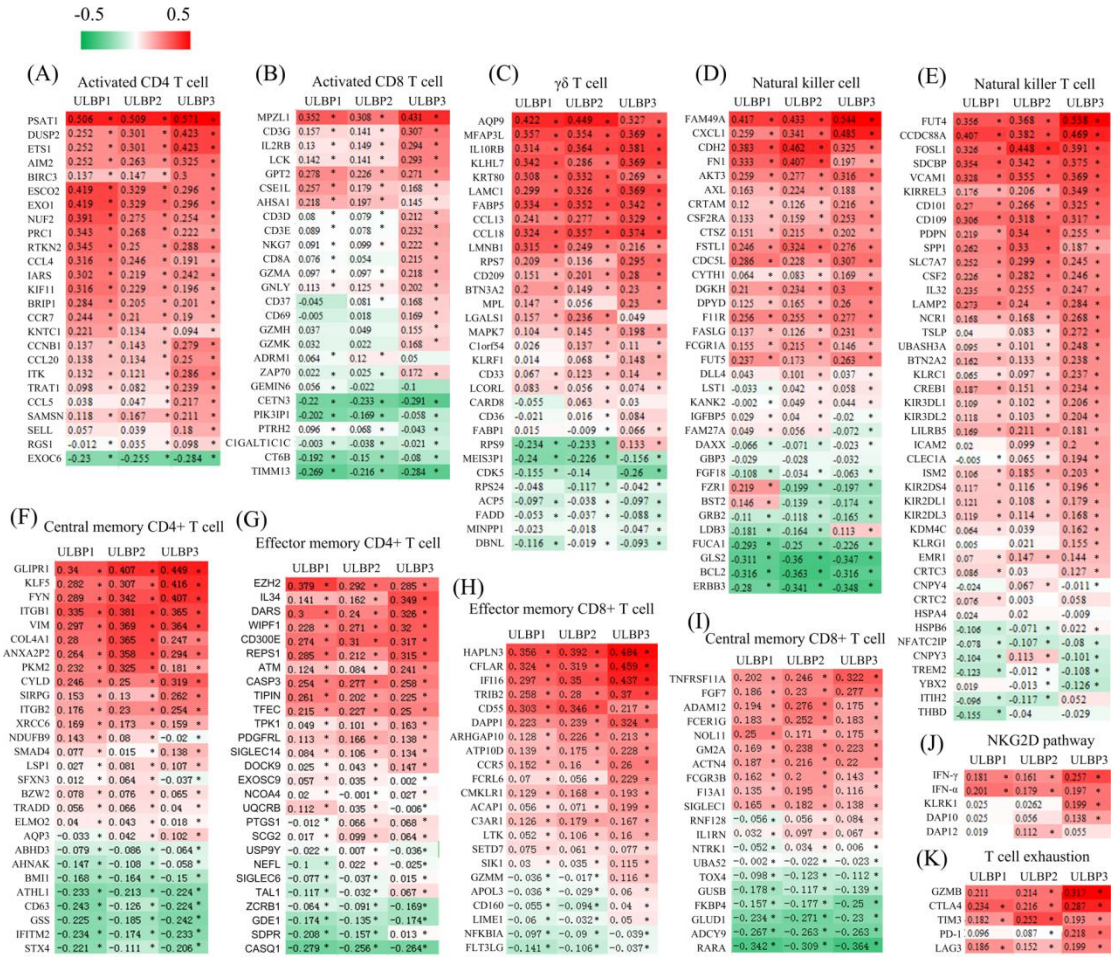


Figure 6 Correlation analysis between ULBP1-3 and immune markers of various immune cells. *, p<0.05

3.7 | *Relatiotnship analysis between ULBP1-3 and five other ligands*

Since the correlations and co-occurrence between ULBP1-3 and five other ligands are largely unknown. Finally, we analyzed the correlations of NKG2DLs with each other by using bc-GenExMiner database. As shown in Figure 7, ULBP1, ULBP2, ULBP3, and ULBP6, which are expressed in glycophosphatidylinositol (GPI)-anchored, are significant and positive correlations with each other. The co-occurrence of NKG2DLs were predicted by using cbioportal. ULBP1-6 were co-occurrence with each other and MIAC was only co-occurrence with MICB, indicating that the genes in the same family were more likely to co-occurrence. While there were no significant mutually exclusive genes.

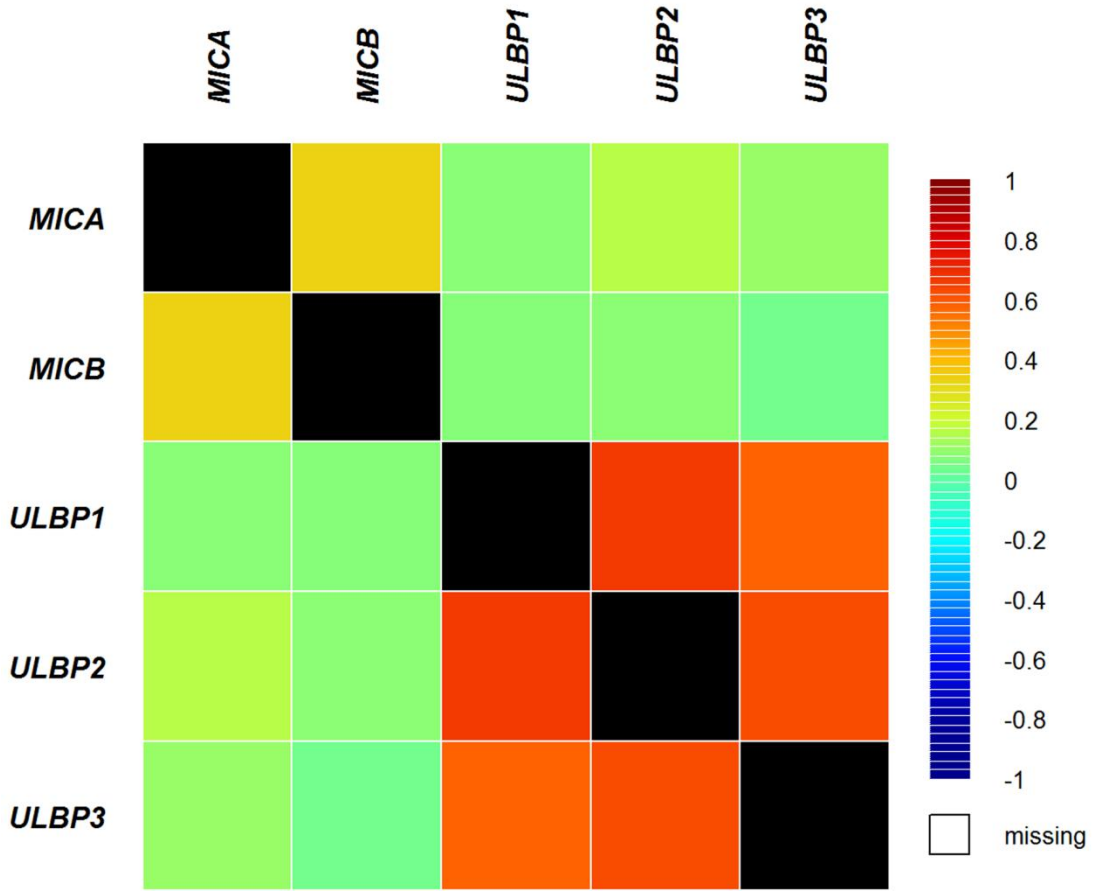


Figure 7 The correlations and interaction between ULBP1-3 and five other ligands

Discussion

NKG2DLs expression is highly heterogeneous, and their expression level can markedly differ depending on the type of cells in response to stress or malignant transformation (Champsaur & Lanier, 2010). Studies have shown that NKG2D ligands are frequently high expression in kinds of cancers such as ovarian cancer, colorectal cancer, and breast cancer (Lazarova & Steinle, 2019). Our research is consistent with the previous findings that ULBP1 and ULBP2 in both mRNA and protein levels of breast cancer patients were higher than that of normal tissues. As regards the expression of ULBP3, gene translation may be widely restricted in normal tissues, thus the protein expression level in breast cancer is higher than that in normal tissue.

Many factors, such as chemotherapeutic agents, miRNA, cytokines, and tumor microenvironment, affect the expression of NKG2DLs in transcriptional, translational, and post-translational modification regulation (Duan et al., 2019; Toledano,

Vitenshtein, Stern-Ginossar, Seidel, & Mandelboim, 2018; Xie et al., 2014). As this particular study shows, the correlation genes of ULBP1-3 enriched in NK cell mediated cytotoxicity, IL-17 signaling pathway, and microRNA in cancer. Meanwhile, Oncogenes directly can induce the expression of NKG2DLs (Boissel et al., 2006; Okita et al., 2012). A study using breast cancer cell lines has confirmed that HER2 overexpression enhanced NK cell-mediated cytotoxicity via inducing the expression of MICA/B (Okita et al., 2012). This contrasts with the present study that HER2+ negatively regulated the expression of ULBP1-3 in patients with breast cancer.

Theoretically, NKG2DLs binding to the activating receptor NKG2D can promote anti-cancer immune responses, with the result that NKG2DLs over-expressed are associated with a good prognosis. However, the prognosis of NKG2DLs changes according to the type of cancer. In cervical cancer, multivariate analysis showed high expression of either ULBP1 or MICA/B and ULBP1 combined resulted in a good prognosis (Cho et al., 2014). de Kruijf et al. demonstrated that MIC-A/B and ULBP-2 expression were independent markers of a longer relapse free period (RFP) in breast cancer patients (de Kruijf et al., 2012). On the contrary, NKG2DLs over-expressed could takes as a poor prognostic factor in ovarian cancer patients (K. Li et al., 2009; McGilvray et al., 2010). However, the survival analysis showed that the high expression of ULBP1 had no significantly effect on the prognosis of breast cancer, whereas the high expression of ULBP2 and ULBP3 predicts an unfavorable prognosis.

Some studies have found the cooperation of NKG2DLs with each other. And the superimposed prognostic effect was observed, when the expression of two NKG2DLs were combined (de Kruijf et al., 2012; McGilvray et al., 2010; McGilvray et al., 2009). Our database analysis showed that the significant correlations and co-occurrence of NKG2DLs occurred in glycophosphatidylinositol (GPI)-anchored form protein and the same family, respectively. So when we consider the additive effect of ligands we should consider their structure and function, don't just think about the union of ligands.

NKG2DLs usually came in two forms: membrane-bound NKG2D ligands (mNKG2DLs), soluble NKG2DLs (sNKG2DLs). Convincing evidence has been found sNKG2DLs are found in serum from patients with kinds of malignancies or in supernatant from various cell lines. mNKG2DLs stimulate tumor immunity by activating cytotoxic T lymphocytes cells and NK cells. While sNKG2DLs compete with mNKG2DLs for NKG2D receptor binding and downregulate effector cell-related NKG2D and eventually impair the effector function of immune cell (Dhar & Wu, 2018). The sNKG2DLs level also associate with tumor stage and down-regulate NKG2D expression on the surface of NK and CD8+ lymphocytes (Groh et al., 2002; Holdenrieder et al., 2006; Jinushi et al., 2008; Salih, Rammensee, & Steinle, 2002; Weil et al., 2017). In addition to the reduction of NKG2D expression, soluble ULBP2 secreted from pancreatic cancer reduced the cytotoxicity of NK (Kegasawa et al., 2019; Song et al., 2006). So ULBP1-3 were positive correlation with markers of multifunctional immune cells on the one hand, on the other hand, the expression level of ULBP1-3 were significantly negative correlation with some markers in NK cells and central memory CD8+ T cells. Thus, the shedding of NKG2DLs not only impair the anti-tumor reactivity of NK but also negative regulate the function of memory of CD8+ T cells. The high expression of ULBP1-3 had no significant influence on the gene expression of NKG2D pathway, it may be also due to the shedding of NKG2DLs.

In conclusion, our data demonstrated that ULBP1-3 up-regulation in breast cancer is correlated with poor prognosis and various markers in immune cells. While the expression of ULBP1-3 were not associated with immune cell infiltration and genes in the NKG2D/NKG2DLs pathway. Besides sharing the same pathway for the regulatory role of ULBP1-3, they also have their own specific pathway. So we should conduct further study to learn the other function of ULBP1-3 in breast cancer.

ACKNOWLEDGMENT

This study was financially supported by the Key Program of National Natural Science Foundation of China (Grant No. 82030076) and National Natural Science Foundation

of China (Grant No. 81670162 and 81470010).

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Dongzhe Liu designed the study and provided the funding. Caixia Han conducted the patients' data and wrote the manuscript. Yutong Zhang and Litao Sun contributed to drafting the article or revising it. Enze Shao analyzed the data. All authors contributed to the article and approved the submission of the manuscript.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available in the Oncomine (http://www.oncomine.org), KM- plotter (http://kmplot.com/analysis/), TIMER (https://cistrome.shinyapps.io/timer/), LinkedOmics (http://www.linkedomics.org/), cbioportal, (http://cbioportal.org), bc-GenExMiner (http://bcgenex.centregauducheau.fr/BC-GEM/GEM-Accueil.php?js=1), and HPA ((http://www.proteinatlas.org/) databases repository.

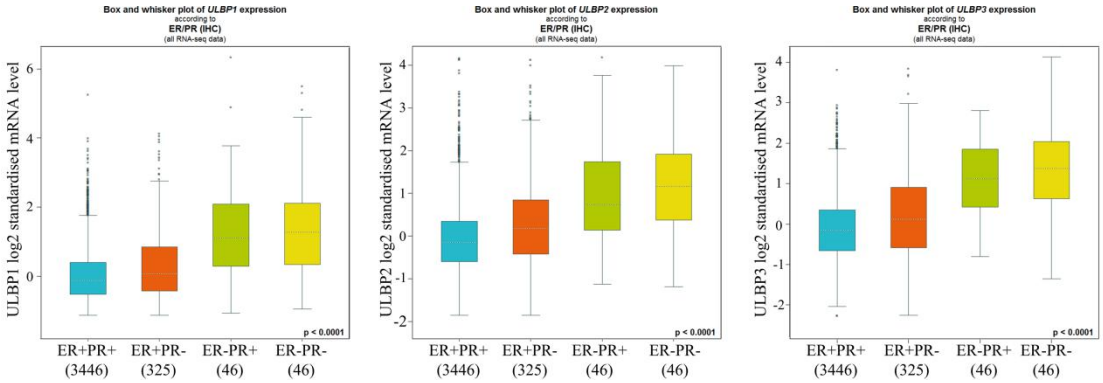
REFERENCES

1. Bajgain, P., Tawinwung, S., D'Elia, L., Sukumaran, S., Watanabe, N., Hoyos, V., . . . Vera, J. F. (2018). CAR T cell therapy for breast cancer: harnessing the tumor milieu to drive T cell activation. *J Immunother Cancer, 6*(1), 34. doi:10.1186/s40425-018-0347-5
2. Baragaño Raneros, A., Martín-Palanco, V., Fernandez, A. F., Rodriguez, R. M., Fraga, M. F., Lopez-Larrea, C., & Suarez-Alvarez, B. (2015). Methylation of NKG2D ligands contributes to immune system evasion in acute myeloid leukemia. *Genes Immun, 16*(1), 71-82. doi:10.1038/gene.2014.58
3. Boissel, N., Rea, D., Tieng, V., Dulphy, N., Brun, M., Cayuela, J. M., . . . Toubert, A. (2006). BCR/ABL oncogene directly controls MHC class I chain-related molecule A expression in chronic myelogenous leukemia. *J Immunol, 176*(8), 5108-5116. doi:10.4049/jimmunol.176.8.5108
4. Champsaur, M., & Lanier, L. L. (2010). Effect of NKG2D ligand expression on host immune responses. *Immunol Rev, 235*(1), 267-285. doi:10.1111/j.0105-2896.2010.00893.x
5. Cho, H., Chung, J. Y., Kim, S., Braunschweig, T., Kang, T. H., Kim, J., . . . Kim, J. H. (2014). MICA/B and ULBP1 NKG2D ligands are independent predictors of good prognosis in cervical cancer. *BMC Cancer, 14*, 957. doi:10.1186/1471-2407-14-957
6. de Kruijf, E. M., Sajet, A., van Nes, J. G., Putter, H., Smit, V. T., Eagle, R. A., . . . Kuppen, P. J. (2012). NKG2D ligand tumor expression and association with clinical outcome in early breast cancer patients: an observational study. *BMC Cancer, 12*, 24. doi:10.1186/1471-2407-12-24

7. Dhar, P., & Wu, J. D. (2018). NKG2D and its ligands in cancer. *Curr Opin Immunol, 51*, 55-61. doi:10.1016/j.coi.2018.02.004
8. Diefenbach, A., Jensen, E. R., Jamieson, A. M., & Raulet, D. H. (2001). Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. *Nature, 413*(6852), 165-171. doi:10.1038/35093109
9. Duan, S., Guo, W., Xu, Z., He, Y., Liang, C., Mo, Y., . . . Wang, F. (2019). Natural killer group 2D receptor and its ligands in cancer immune escape. *Mol Cancer, 18*(1), 29. doi:10.1186/s12943-019-0956-8
10. Groh, V., Wu, J., Yee, C., & Spies, T. (2002). Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature, 419*(6908), 734-738. doi:10.1038/nature01112
11. Holdenrieder, S., Stieber, P., Peterfi, A., Nagel, D., Steinle, A., & Salih, H. R. (2006). Soluble MICB in malignant diseases: analysis of diagnostic significance and correlation with soluble MICA. *Cancer Immunol Immunother, 55*(12), 1584-1589. doi:10.1007/s00262-006-0167-1
12. Jelenčić, V., Lenartić, M., Wensveen, F. M., & Polić, B. (2017). NKG2D: A versatile player in the immune system. *Immunol Lett, 189*, 48-53. doi:10.1016/j.imlet.2017.04.006
13. Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J., & Thun, M. J. (2007). Cancer statistics, 2007. *CA Cancer J Clin, 57*(1), 43-66. doi:10.3322/canjclin.57.1.43
14. Jinushi, M., Vanneman, M., Munshi, N. C., Tai, Y. T., Prabhala, R. H., Ritz, J., . . . Dranoff, G. (2008). MHC class I chain-related protein A antibodies and shedding are associated with the progression of multiple myeloma. *Proc Natl Acad Sci U S A, 105*(4), 1285-1290. doi:10.1073/pnas.0711293105
15. Kegasawa, T., Tatsumi, T., Yoshioka, T., Suda, T., Ikezawa, K., Nakabori, T., . . . Takehara, T. (2019). Soluble UL16-binding protein 2 is associated with a poor prognosis in pancreatic cancer patients. *Biochem Biophys Res Commun, 517*(1), 84-88. doi:10.1016/j.bbrc.2019.07.020
16. Kohga, K., Takehara, T., Tatsumi, T., Miyagi, T., Ishida, H., Ohkawa, K., . . . Hayashi, N. (2009). Anticancer chemotherapy inhibits MHC class I-related chain a ectodomain shedding by downregulating ADAM10 expression in hepatocellular carcinoma. *Cancer Res, 69*(20), 8050-8057. doi:10.1158/0008-5472.Can-09-0789
17. Lánczky, A., Nagy, Á., Bottai, G., Munkácsy, G., Szabó, A., Santarpia, L., & Győrffy, B. (2016). miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. *Breast Cancer Res Treat, 160*(3), 439-446. doi:10.1007/s10549-016-4013-7
18. Lazarova, M., & Steinle, A. (2019). The NKG2D axis: an emerging target in cancer immunotherapy. *Expert Opin Ther Targets, 23*(4), 281-294. doi:10.1080/14728222.2019.1580693
19. Li, K., Mandai, M., Hamanishi, J., Matsumura, N., Suzuki, A., Yagi, H., . . . Konishi, I. (2009). Clinical significance of the NKG2D ligands, MICA/B and ULBP2 in ovarian cancer: high expression of ULBP2 is an indicator of poor prognosis. *Cancer Immunol Immunother, 58*(5), 641-652. doi:10.1007/s00262-008-0585-3
20. Li, T., Fan, J., Wang, B., Traugh, N., Chen, Q., Liu, J. S., . . . Liu, X. S. (2017). TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. *Cancer Res, 77*(21), e108-e110. doi:10.1158/0008-5472.Can-17-0307

21. McGilvray, R. W., Eagle, R. A., Rolland, P., Jafferji, I., Trowsdale, J., & Durrant, L. G. (2010). ULBP2 and RAET1E NKG2D ligands are independent predictors of poor prognosis in ovarian cancer patients. *Int J Cancer, 127*(6), 1412-1420. doi:10.1002/ijc.25156
22. McGilvray, R. W., Eagle, R. A., Watson, N. F., Al-Attar, A., Ball, G., Jafferji, I., . . . Durrant, L. G. (2009). NKG2D ligand expression in human colorectal cancer reveals associations with prognosis and evidence for immunoediting. *Clin Cancer Res, 15*(22), 6993-7002. doi:10.1158/1078-0432.Ccr-09-0991
23. Morisaki, T., Onishi, H., Koya, N., Kiyota, A., Tanaka, H., Umebayashi, M., . . . Katano, M. (2011). Combinatorial cytotoxicity of gemcitabine and cytokine-activated killer cells in hepatocellular carcinoma via the NKG2D-MICA/B system. *Anticancer Res, 31*(7), 2505-2510.
24. Ohashi, M., Eagle, R. A., & Trowsdale, J. (2010). Post-translational modification of the NKG2D ligand RAET1G leads to cell surface expression of a glycosylphosphatidylinositol-linked isoform. *J Biol Chem, 285*(22), 16408-16415. doi:10.1074/jbc.M109.077636
25. Okita, R., Mougiakakos, D., Ando, T., Mao, Y., Sarhan, D., Wennerberg, E., . . . Kiessling, R. (2012). HER2/HER3 signaling regulates NK cell-mediated cytotoxicity via MHC class I chain-related molecule A and B expression in human breast cancer cell lines. *J Immunol, 188*(5), 2136-2145. doi:10.4049/jimmunol.1102237
26. Paczulla, A. M., Rothfelder, K., Raffel, S., Konantz, M., Steinbacher, J., Wang, H., . . . Lengerke, C. (2019). Absence of NKG2D ligands defines leukaemia stem cells and mediates their immune evasion. *Nature, 572*(7768), 254-259. doi:10.1038/s41586-019-1410-1
27. Rhodes, D. R., Kalyana-Sundaram, S., Mahavisno, V., Varambally, R., Yu, J., Briggs, B. B., . . . Chinnaiyan, A. M. (2007). Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia, 9*(2), 166-180. doi:10.1593/neo.07112
28. Ru, B., Wong, C. N., Tong, Y., Zhong, J. Y., Zhong, S. S. W., Wu, W. C., . . . Zhang, J. (2019). TISIDB: an integrated repository portal for tumor-immune system interactions. *Bioinformatics, 35*(20), 4200-4202. doi:10.1093/bioinformatics/btz210
29. Salih, H. R., Rammensee, H. G., & Steinle, A. (2002). Cutting edge: down-regulation of MICA on human tumors by proteolytic shedding. *J Immunol, 169*(8), 4098-4102. doi:10.4049/jimmunol.169.8.4098
30. Song, H., Kim, J., Cosman, D., & Choi, I. (2006). Soluble ULBP suppresses natural killer cell activity via down-regulating NKG2D expression. *Cell Immunol, 239*(1), 22-30. doi:10.1016/j.cellimm.2006.03.002
31. Spear, P., Wu, M. R., Sentman, M. L., & Sentman, C. L. (2013). NKG2D ligands as therapeutic targets. *Cancer Immun, 13*, 8.
32. Stanton, S. E., & Disis, M. L. (2016). Clinical significance of tumor-infiltrating lymphocytes in breast cancer. *J Immunother Cancer, 4*, 59. doi:10.1186/s40425-016-0165-6
33. Stojanovic, A., Correia, M. P., & Cerwenka, A. (2018). The NKG2D/NKG2DL Axis in the Crosstalk Between Lymphoid and Myeloid Cells in Health and Disease. *Front Immunol, 9*, 827. doi:10.3389/fimmu.2018.00827
34. Thul, P. J., & Lindskog, C. (2018). The human protein atlas: A spatial map of the human proteome. *Protein Sci, 27*(1), 233-244. doi:10.1002/pro.3307
35. Toledano, T., Vitenshtein, A., Stern-Ginossar, N., Seidel, E., & Mandelboim, O. (2018). Decay of the Stress-Induced Ligand MICA Is Controlled by the Expression of an Alternative 3' Untranslated Region. *J Immunol, 200*(8), 2819-2825. doi:10.4049/jimmunol.1700968

36. Vasaikar, S. V., Straub, P., Wang, J., & Zhang, B. (2018). LinkedOmics: analyzing multi-omics data within and across 32 cancer types. *Nucleic Acids Res, 46*(D1), D956-d963. doi:10.1093/nar/gkx1090
37. Weil, S., Memmer, S., Lechner, A., Huppert, V., Giannattasio, A., Becker, T., . . . Koch, J. (2017). Natural Killer Group 2D Ligand Depletion Reconstitutes Natural Killer Cell Immunosurveillance of Head and Neck Squamous Cell Carcinoma. *Front Immunol, 8*, 387. doi:10.3389/fimmu.2017.00387
38. Xie, J., Liu, M., Li, Y., Nie, Y., Mi, Q., & Zhao, S. (2014). Ovarian tumor-associated microRNA-20a decreases natural killer cell cytotoxicity by downregulating MICA/B expression. *Cell Mol Immunol, 11*(5), 495-502. doi:10.1038/cmi.2014.30



Supplemental Figure1 ULBP1-3 expression in ER and PR combinations

Figures

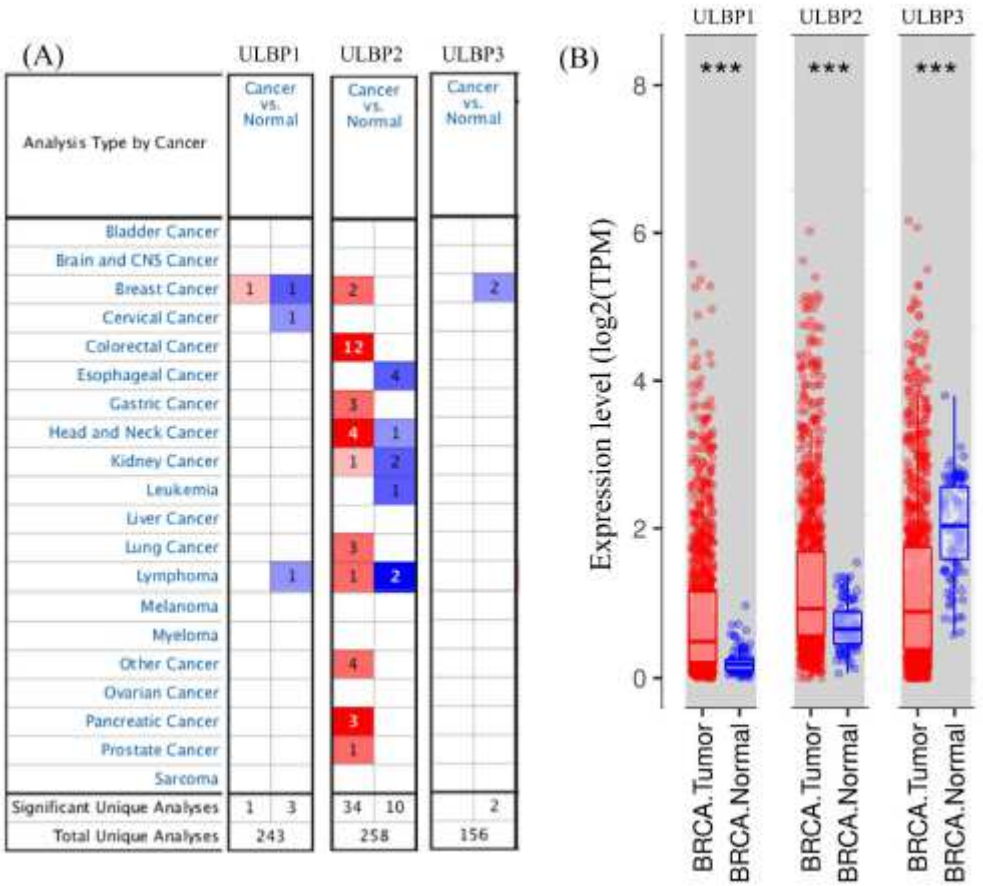


Figure 1

ULBP1, ULBP2, and ULBP3 expression in breast cancer and normal tissues. (A), NKG2DLs expression in the Oncomine database. (B), ULBP1, ULBP2, and ULBP3 expression expression in the TIMER databas

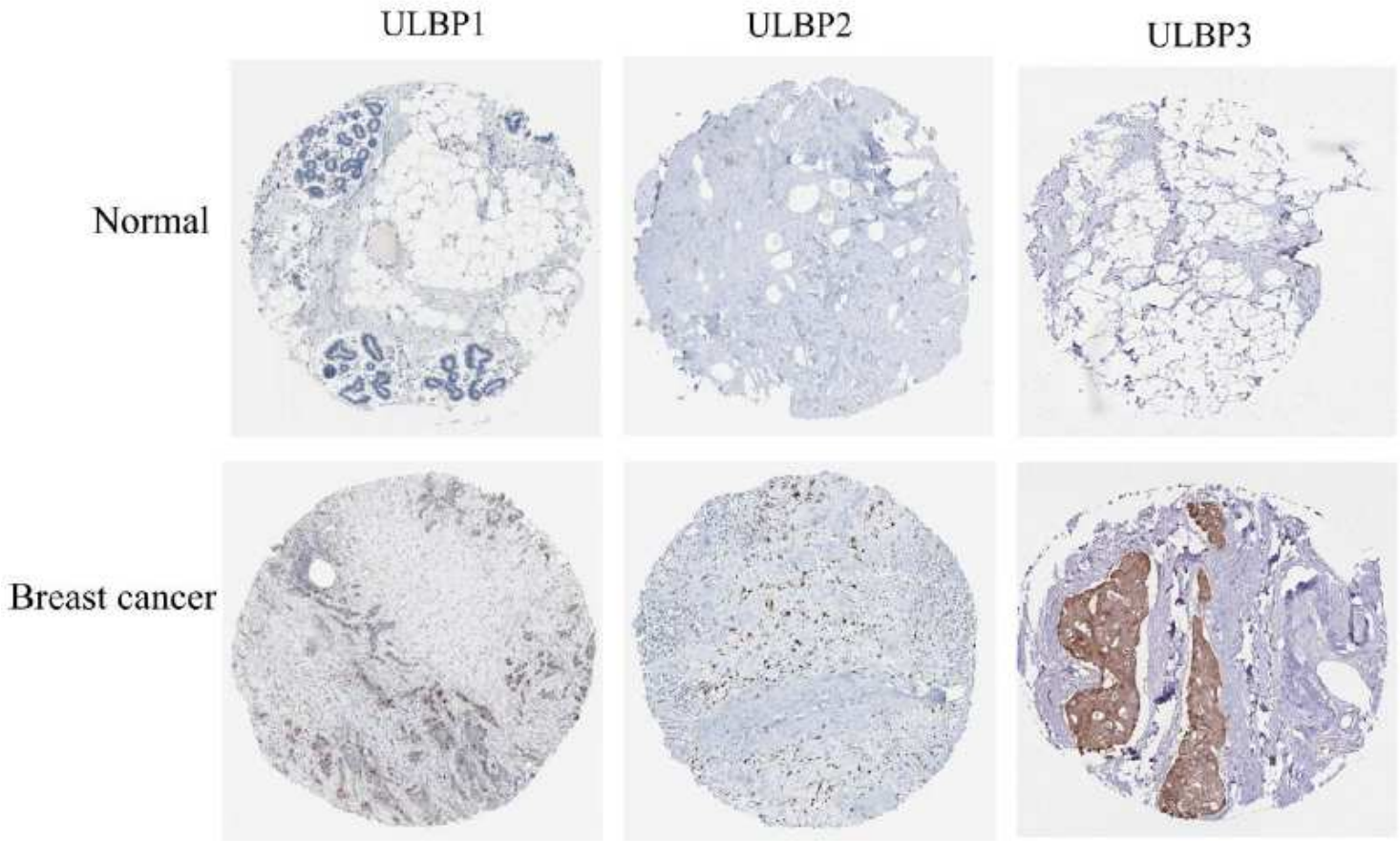


Figure 2

IHC images of ULBP1, ULBP2, and ULBP3 detected in the HPA

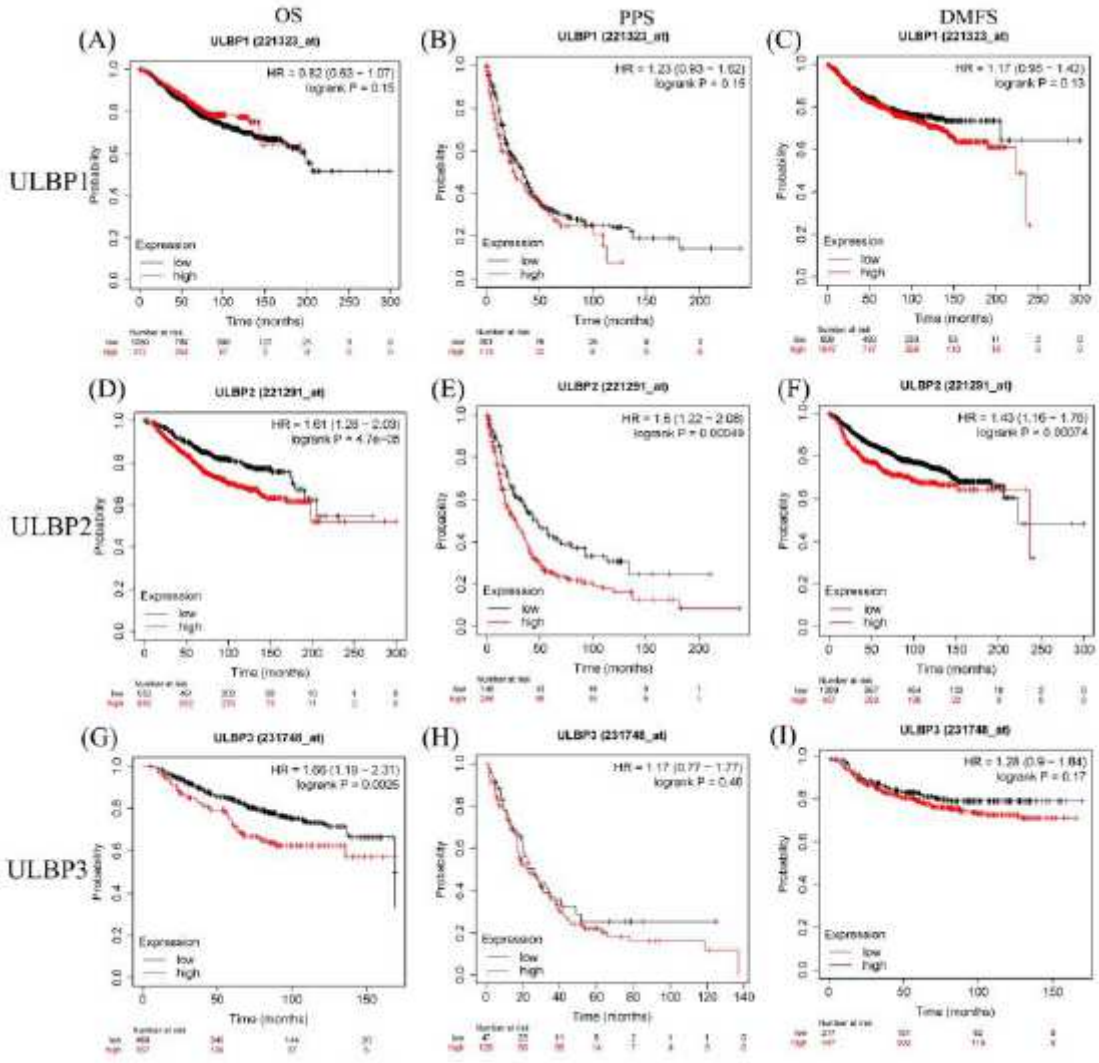


Figure 3

Clinical significance of ULBP1-3 in breast cancer patients. (A-C), Survival curves of OS, PPS, and DMFS for ULBP1 in breast cancer, respectively; (D-F), Survival curves of OS, PPS, and DMFS for ULBP2 in breast cancer, respectively; (G-I), Survival curves of OS, PPS, and DMFS for ULBP2 in breast cancer, respectively

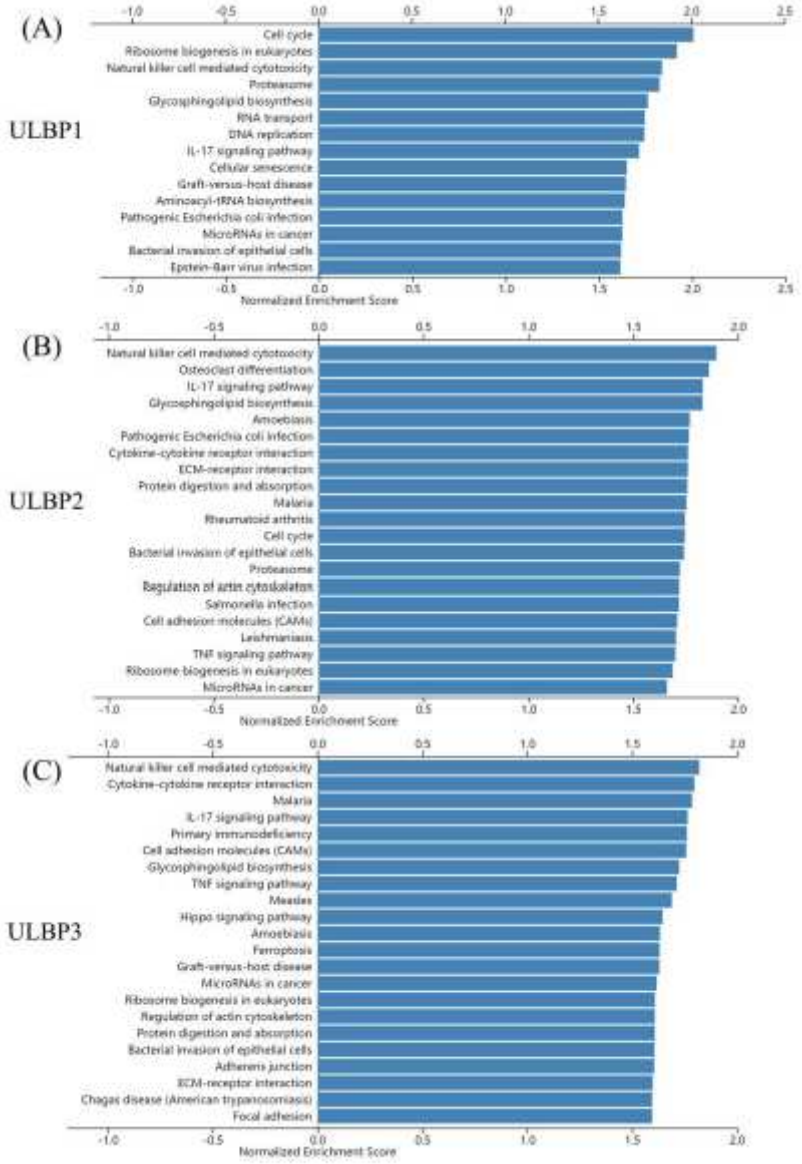


Figure 4

Significantly enriched KEGG pathways of ULBP1-3 in breast cancer patients (FDR<0.05). (A), KEGG pathways of ULBP1; (B), KEGG pathways of ULBP2; (C), KEGG pathways of ULBP3

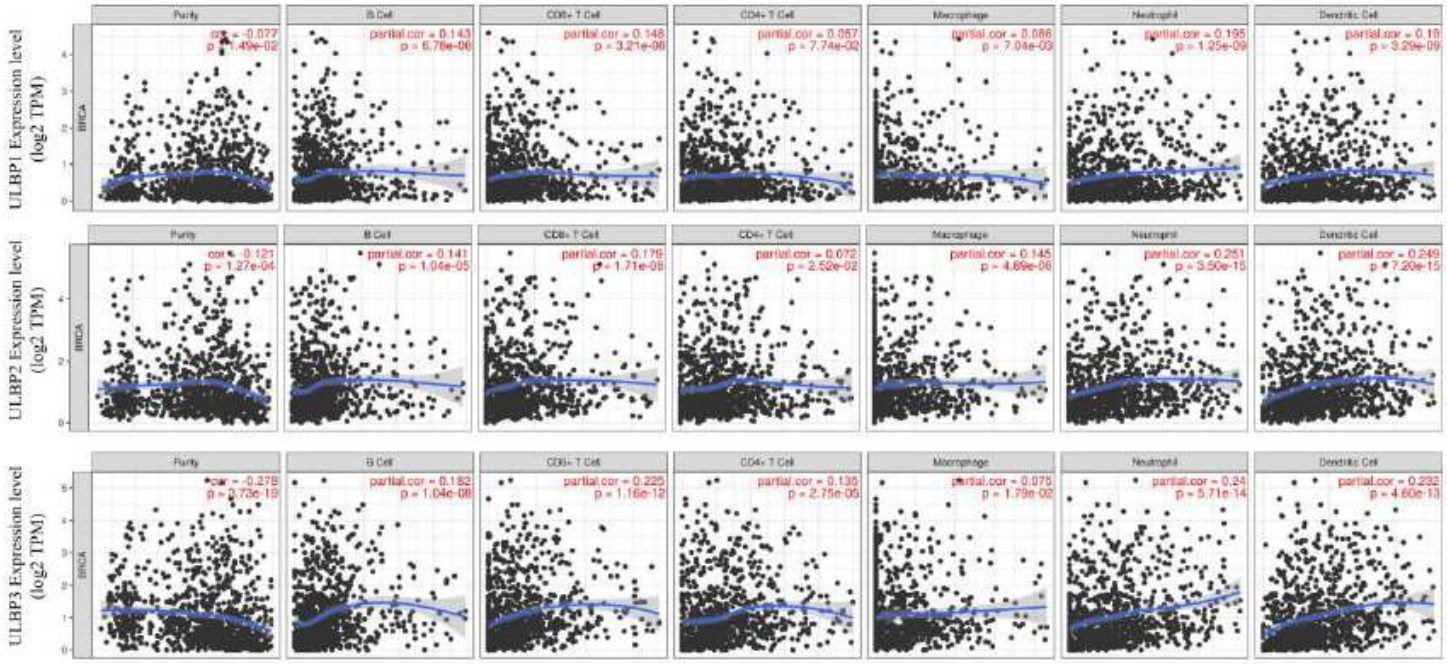


Figure 5

Correlation of NKG2DLs expression with immune infiltration level in breast cancer cohort

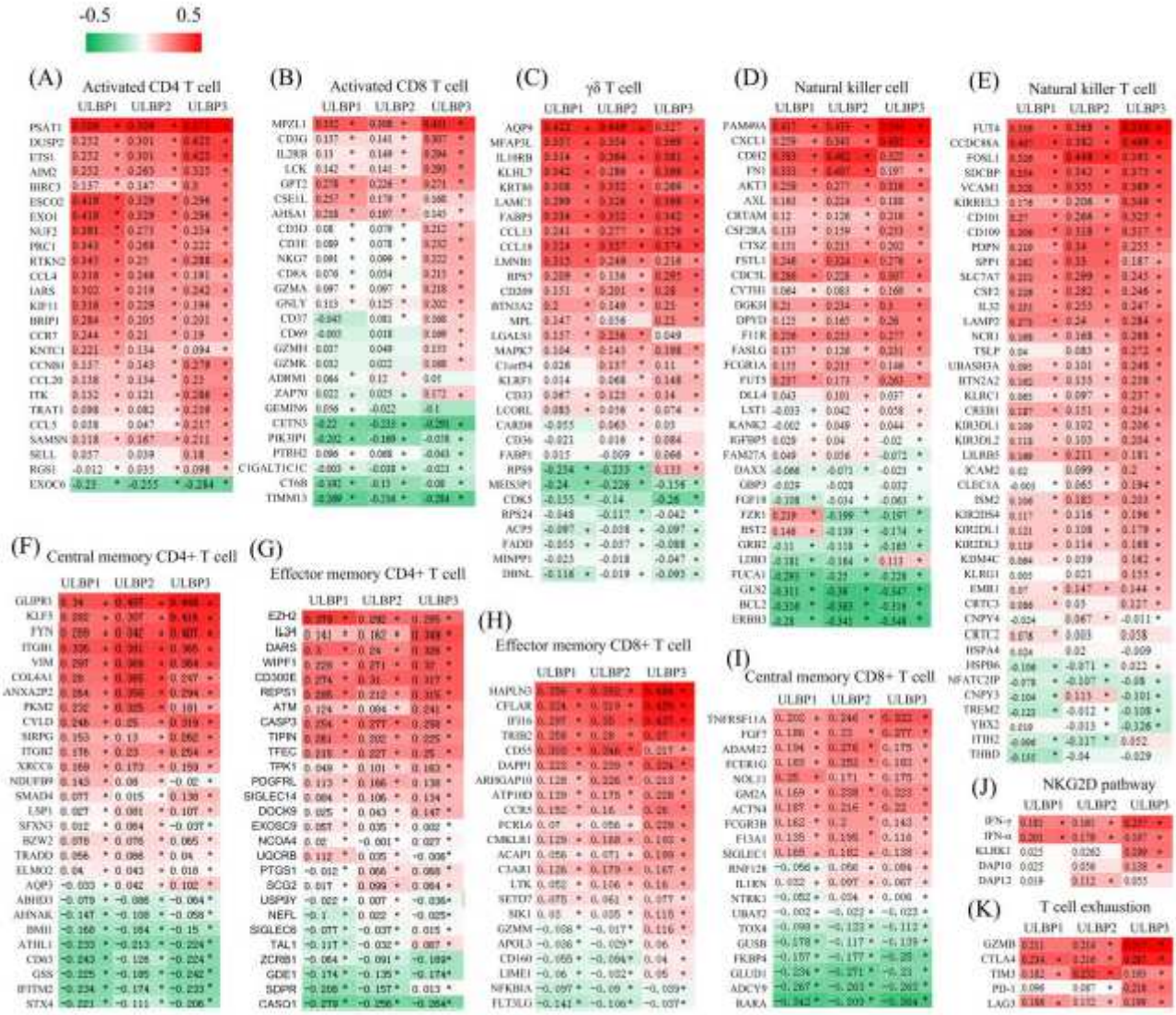


Figure 6

Correlation analysis between ULBP1-3 and immune markers of various immune cells. *, p<0.05

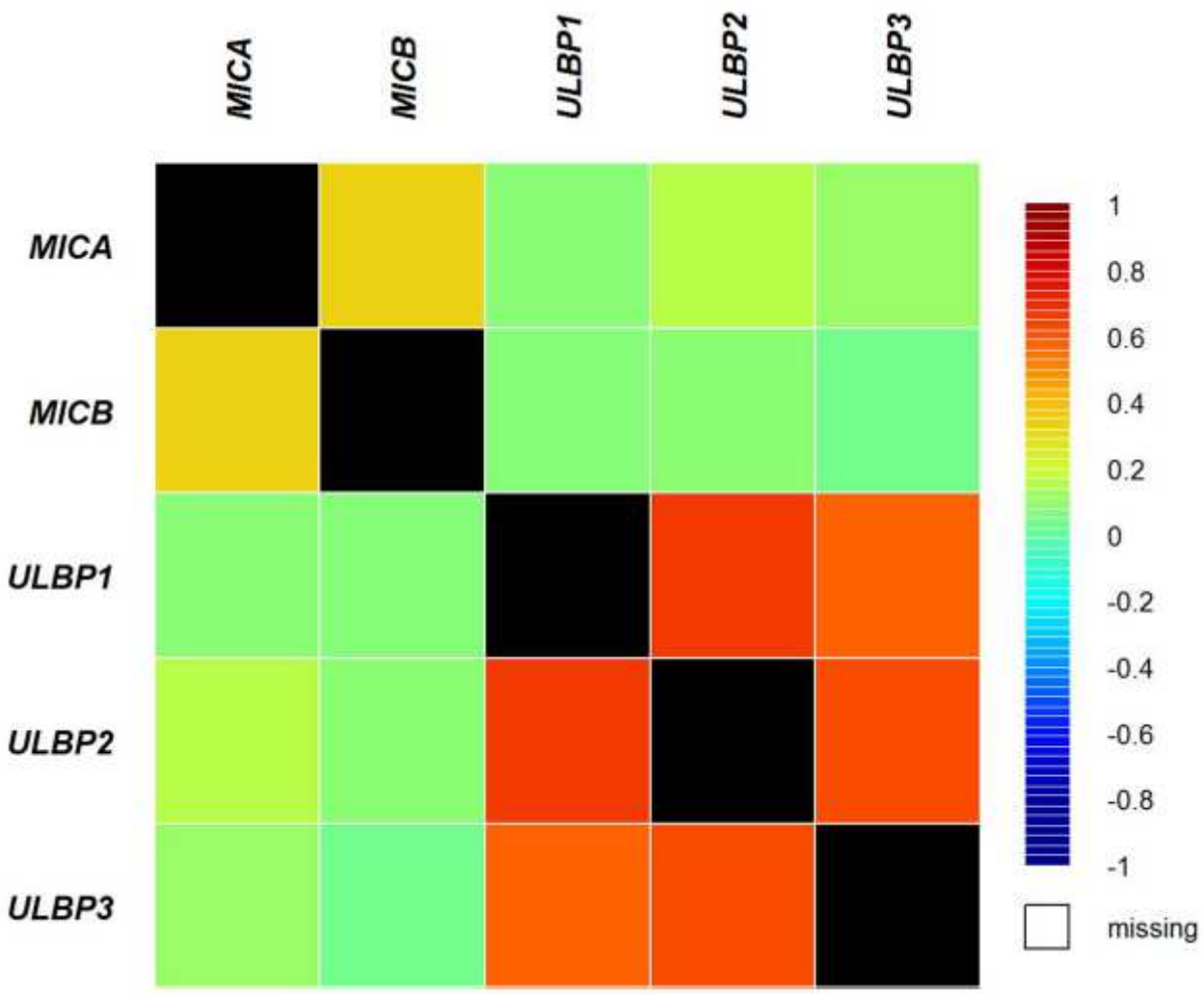


Figure 7

The correlations and interaction between ULBP1-3 and five other ligands

Supplementary Files

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- FigS1.png