Supplementary Online Content

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eMethods 1. Collecting NK cell-specific (NK-specific) Genes

To select NK-specific genes, we used the gene signatures derived from a genome-wide gene expression analysis of mouse lymphocytes between immune cells\(^1\) and an immune gene regulatory network (ImmGen)\(^2\) containing the gene expression profiles of more than 300 mouse immune cell types. NK cells were reported to be easily distinguished from B cells and adaptive T cells such as CD4\(^+\) T, CD8\(^+\) T and other innate immune cells but shared many up-regulated genes with NKT and \(\gamma\delta\) T cells.\(^1\)

The shared repertoire of surface receptors, signaling molecules and transcription factors expressed by NK cells and the innate-like T cells (i.e., NKT and \(\gamma\delta\) T cells) blurs the distinctions among these cell types. Thus, we collected \(~200\) NK-specific genes representing (1) NK-unique genes which were more highly up-regulated in NK cells than innate-like T cells (i.e., NKT and \(\gamma\delta\) T cells) and other immune cells (i.e., B, CD4\(^+\) T, CD8\(^+\) T and others); (2) genes (termed as NK-NKT genes here) which were highly up-regulated in both NK and NKT cells, but not in other immune cells; (3) genes (i.e., termed as NK-\(\gamma\delta\) T genes here) which were highly up-regulated in both NK and \(\gamma\delta\) T cells, but not in other immune cells; and (4) genes (i.e., termed as NK-NKT-\(\gamma\delta\) T genes here) which were highly up-regulated in NK, NKT and \(\gamma\delta\) T cells, but not in other immune cells. These genes were able to distinguish innate populations (NK, NKT and \(\gamma\delta\) T cells) from adaptive T cells, B cells and other immune cells.

We manually checked these genes from literature and gene databases such as GeneCards (https://www.genecards.org) to remove the genes which were universally expressed. Furthermore, we conducted correlation analyses between the expression of each gene and the abundance of TIL-NK, TIL-NK CD56\(^{bright}\) or TIL-NK CD56\(^{dim}\) cells in each cancer type. For each cancer type, the genes were remained only if they had significantly positive correlations with the abundance of any of the three TIL-NK cell subsets (FDR-corrected \(p<0.05\)). Finally, we obtained 157 genes (i.e., 28 of them were NK-unique genes). These genes were highly expressed on either NK cell alone, NK-NKT, NK-\(\gamma\delta\) T or NK-NKT-\(\gamma\delta\) T cells. To exclude the genes that have a
functional effect on tumor cells, we further screened the genes (i.e., from the 157 gene) which were within the bottom 30% of the expression value-ranked whole-genome genes in the TIME-rich tumors.

**eMethods 2. Immunoreceptor Tyrosine-Based Activation Motif (ITAM)-Signaling Genes**

Besides the NK cell specific ITAM-signaling receptors, we manually collected 18 ITAM-signaling genes (eTable 8) from literature which were expressed in NK cells and other immune cells such as T cells.

**eMethods 3. Ligands of the NK Cell Activating Receptors**

We manually collected 39 ligands from literature which were known as NK cell activating receptors (eTable 9).

**eMethods 4. Variant Calling and Functional Germline Variants**

For WES files, variant calling was performed using Varscan (version-2.3.9)\(^3\). Functional variants were examined and annotated using the Combined Annotation Dependent Depletion (CADD)\(^4\) with the default parameters. To consistent with the WES data analysis pipeline in TCGA, BWA (version-0.7.15)\(^5\) was used to align with default parameters for the cancer-free individuals, and pipe into Samtools (version-0.1.8)\(^6\) to sort. Additional adding read groups and duplicate removal were processed with Picard-tools (version-2.6.0). The resulted BAM files were processed with GATK (version-4.0.11.0) for realignment and base recalibration.
Immune-related genes (n=1,384) including MHC system-related genes, immunophenoscore-related genes, ICT essential genes for immunotherapy and cytotoxic T cell-resistant genes were collected and identified as critical immune-related genes (the gene pool \( G \)). RNA-sequencing data of melanoma samples were used to conduct the following analysis:

Step#1 Initialize the candidate set of key genes, that is, \( G_{\text{candidate}} = \emptyset \)

Step#2 Randomly select 30% genes from the gene set \( G_{\text{random}} \).

Step#3 Replace the features of elements in the patient set \( P \) with \( G_{\text{random}} \) to form the sample set \( S_{\text{random}} \).

Step#4 Group the samples \( S_{\text{random}} \) by using the hierarchical clustering method. For each of the clustering, clValid\(^{11}\) was used to evaluate the clustering stability and the most stable clustering number was recorded.

Step#5 Repeat Steps 2-4 100,000 times. Rank the most stable clustering numbers and select the most suitable clustering number 3.

Step#6 Extract the genes when clustering number is 3 and rank the genes.

Select the most informative genes and record them as the final set of key-gene candidates (1,294 genes).

We then used the 1,294 genes to conduct unsupervised clustering analyses of the RNA-seq data for each cancer type to define TIME subtypes.
eMethods 6. Assigning Immune Checkpoint Therapy (ICT) Trial Samples into TIME Subtypes

ICT-clinical trial SKCM and STAD samples were assigned to the TIME subtypes derived from TCGA-SKCM and TCGA-STAD samples, respectively. To assign an ICT-clinical trial sample into a TIME subtype, t-test statistics were first conducted between TIME-rich and TIME-intermediate tumors, and between TIME-intermediate and TIME-poor tumors based on the genes derived from the NK cell-mediated cytotoxicity pathway and the Wnt signaling pathway using RNA-seq data. Spearman's correlation was then conducted between each ICT-clinical trial sample and the TCGA samples in each TIME subtype based on the obtained significantly differential genes (p<0.05). Finally, k-nearest neighbor algorithm (KNN, k=5) was used to determine the subtype of each ICT-clinical trial sample.

eMethods 7. Randomization Tests of the NK-Defective Genes

For each cancer type, we identified a set of NK-defective genes. To test if a set of NK-defective genes could be randomly identified, we conducted randomization tests. We first randomly selected 157 genes within the bottom 30% of the expression value-ranked whole-genome genes of the TIME-rich tumors for a given cancer type (n=5,000 times) and then employed hypergeometric tests. When a negative correlation (p<0.05) between the gene number in the random genes and the abundance of TIL-NK cells appeared, the hypergeometric test was marked as one success. The number of successes was calculated within the 10,000 hypergeometric tests.

eMethods 8. Validating the Observation that Inherited Defective Genes in NK Cells and APP Pathway Were More in Patients With Cancer Than Individuals With No Cancer

To further validate the observation that more inherited defective genes in NK cells were in cancer patients than cancer-free individuals, we obtained the WES data of germlines for 12,380 cancer-free samples from dbGaP (phs000473) and determined their functional mutated genes. For each cancer type, we conducted hypergeometric
tests for the NK cell-mediated cytotoxicity pathway, NK cell-associated phenotypes and the APP pathway shown in Figure 3 and eFigure 4 using the methods in our previous study. Briefly, for a given cancer type, we compared cancer hallmark genes (~12,000 genes) using randomization tests to identify differentially functional germline mutated genes (p<0.05) between cancer and the cancer-free samples, and then conducted enrichment analyses of the NK cell-mediated cytotoxicity pathway, NK cell-associated phenotypes and APP pathway via conducting hypergeometric tests (differentially functional germline mutated genes vs the cancer hallmark genes).

eAppendix 1. Associations of Defective Genes in NK Cell-ITAM-Signaling Genes With Clinical Outcomes and Abundance of TILs

To test whether ITAM signaling-genes in NK cells could have more inherited defects in TIME-poor tumors compared with TIME-rich tumors, we collected a set of known ITAM-dependent genes (~20 genes) in NK cells and found that some of the genes had significantly higher genetic defects in TIME-poor tumors than TIME-rich tumors (eTable 5). We further asked whether the combination of these significantly defective genes with the potentially NK cell-defective genes identified in each cancer type could improve the correlations (i.e., the negative correlations between the number of NK cell-defective genes and survival and TILs’ abundance). We showed that the correlations were significantly improved (i.e., lower p values and correlation coefficients) in 11 out of the 12 cancer types except COAD. These results suggested that inheritably genetic defects in ITAM-signalling genes of the NK cells could highly associated with TILs’ abundance and survival in all the cancers except COAD in a synergy manner. NK cells surrounding COAD tumors enabled to directly interact with environmental factors such as microbiome, drinks, and others so that COAD associated NK cells were much more complex than other cancers.

eAppendix 2. Inherited Defective Genes in NK cells, Type I Diabetes, Long-term Depression Phenotypes, and Cancer
Germline genomic analysis here suggested that Type I diabetes and long-term depression phenotypes were linked to some cancers. This is supported by non-genetic studies showing that diabetes is a risk factor for all-site cancer through a meta-analysis of 121 cohorts including 20 million individuals and one million events. A 24-year follow-up study showed that depression increases the risk of cancer, moreover, a meta-analysis of 16 studies (n=163,000) showed that cancer patients with anxiety and depression had a greater risk of dying from all types of cancer. The research implied that the impairment of NK cell function is probably one of the common factors behind these links. For example, obesity has been known to impair NK cell function and then lead to an increased risk for severe infections and several cancer types, while chronic family stress is consistently associated with decreases in NK cell cytotoxicity. These results indicated that NK function impaired by either genetic defects or regulatory factors could increase cancer incidence.

**eAppendix 3. Associations of the Inherited Defective Genes in APP and Wnt Pathways With Tumorigenesis and Metastasis**

It has been shown that activated Wnt signaling pathway in tumors excluded the recruitment of CD8+ T cells into TIMEs. Also, somatic mutations of the Wnt pathway in tumors could activate it to prevent T cells from being recruited into TIMEs. Here we showed that in most of the cancer types the number of inherited defective genes in the Wnt pathway had a positive correlation with the gene expression of the Wnt pathway (i.e., pathway activation) in their paired tumors. In addition, the number of the inheritably defective genes in the Wnt pathway showed weakly negative correlations (i.e., with marginally significant p values in the range of 0.02-0.05) with the clinical outcomes for 8 cancer types (i.e., BLCA, BCRA, HNSC, LAUD, LUSC, PRAD, STAD and UCEC, eFigure 17). Also, the number of the inheritably defective genes in the Wnt pathway had negative correlations with the abundance of TILs in 6 cancer types (i.e., HNSC, KIRC, LGG, LUAD, LUSC and SKCM, eFigure 18) although the correlations were weaker (i.e., in terms of correlation co-efficiencies and correlation significance represented by p values) than
those derived from defective NK cells. These results suggested the association between inherited dysregulations in Wnt pathway and TILs’ recruitment in some cancer types, and its influences were much weaker than defective NK cells.

APP is a biological process which presents antigens to T cells, functional defects in APP could lead tumor cells to escape T cell surveillance. However, careful analyses in this study showed that the number of genetic defective genes in the APP pathway (i.e., the KEGG APP pathway includes HLA family genes, TAP1/2 and other genes) was not significantly associated with clinical outcomes and TILs’ abundance. However, significantly more inherited defective genes in the APP pathway were observed in cancer patients than cancer-free individuals in most cancers (Figure 5 in the main text). These results suggested that individuals who bear more inherited defective genes in the APP pathway probably were at-risk of developing cancers. These insights provided a potential opportunity to identify the subpopulation at-risk of developing cancers based on the inherited defective genes in NK cells and the APP pathway.

eAppendix 4. Open Questions Remained for NK Cell Inherited Defective Genes in Cancer

In this study, we showed that inherited defective genes in NK cells shaped TIME subtypes, TILs’ abundance, survival and cancer risk. Along this line, many open questions still remained, for example, defective genes in NK cells were largely shared by different cancer types, however, each cancer type has a few unique NK-defective genes. Given the fact that tissue/organ-resident NK cells are different and diverse,21 additional studies will be needed to elucidate if defective genes are dominantly expressed in tissue/organ-resident NK cells. If so, adoptive transfer of tissue-resident NK cells could be considered to be more efficient in cancer prevention and TIL's recruitment. NK cells share gene expression programs with NKT and γδ T cells, each of which is a subset of innate-like T cells. NK cells, γδ T cells and NKT cells are cytotoxic cells, which trigger innate immune responses, provide the first level of
defense against infected cells and tumor cells, produce cytokines and trigger immune responses without a prior sensitization by the immune system. Along with this point, we hypothesized that inherited defective genes in NKT and γδ T cells could also play important roles which were discussed in this study, although the cell number of the NK cells is nearly 200 times of that of the NKT and γδ T cells in periphery blood. Genetic defects in NK-NKT and NK-γδ T genes could impair the functions of not only NK cells but also NTK or γδ T cells, therefore, another possibility is that NK, NKT and γδ T cell impairment could contribute together to the results presented in this study. If this is the case, genome-editing to correcting the genetic defective genes in patients’ hematopoietic stem cells which generate NK, NKT, γδ T and other immune cells could be an option for improving existing immunotherapies and cancer prevention for high-risk individuals.
eFigure 1. Heatmaps Showing the 3 Universal TIME Subtypes
Heatmaps showing the three universal TIME subtypes derived from the unsupervised clustering by using the expression of the immune-checkpoint therapy essential genes. Columns and rows represent samples and genes, respectively. Cancer types include bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), kidney renal clear cell carcinoma (KIRC), lower grade glioma (LGG), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PRAD), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA) and uterine corpus endometrial carcinoma (UCEC). Red, green and blue bars represent TIME-rich, TIME-intermediate and TIME-poor subtype, respectively.
eFigure 2. Abundance of the Tumor-Infiltrating Lymphocytes in TIME Subtypes in Cancers
Rows represent tumor-infiltrating lymphocytes.
eFigure 3. Kaplan-Meier Curves Between Patients With Cancer in TIME-Rich Subtype and TIME-Intermediate and TIME-Poor Subtypes.

It revealed that survival for was significantly longer for TIME-rich patients than TIME-intermediate/-poor patients. A substantial fraction of samples in COAD was virus-infected tumors After removing them, only 87 COAD samples were remained for analysis.
**eFigure 4.** Significantly Enriched Pathways by Comparing RNA-seq Data in TIME-Intermediate and TIME-Poor Subtypes

Columns and rows represent KEGG pathways and cancer types, respectively. The digital numbers represent FDR-corrected p values.
**eFigure 5.** Heatmaps of the Significantly Differential Functional Germline Variants Between the TIME-Rich and TIME-Intermediate/TIME-Poor Subtypes (Fisher's exact tests with FDR-corrected p<0.25). Only the functional germline variants (rows) which were less enriched in the TIME-rich subtype are shown. Red, green and blue bars represent TIME-rich, TIME-intermediate and TIME-poor subtype, respectively. Columns represent samples.
**eFigure 6.** Significantly Enriched Pathways by Comparing Functional Germline Variant Between TIME-Rich and TIME-Intermediate/TIME-Poor Subtypes

Columns and rows represent KEGG pathways and cancer types, respectively. The digital numbers represent FDR-corrected p values. Significantly functional differential germline variants \((p<0.2)\) between TIME-rich and TIME-intermediate/-poor tumors were intersected with the immune-checkpoint therapy (ICT) essential genes, and then pathway enrichment analysis was conducted.

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eFigure 7. Significantly Enriched Pathways of the Significantly Differential Germline Variants Between the TIME-Intermediate and TIME-Poor Subtypes

A heatmap showing the significantly enriched pathways derived from the significantly differential germline variants between TIME-intermediate and TIME-poor subtypes. Columns and rows represent KEGG pathways and cancer types, respectively. The digital numbers represent FDR-corrected p values. Significantly functional differential germline variants (p<0.2) between TIME-intermediate and TIME-poor tumors were intersected with the immune-checkpoint therapy (ICT) essential genes, and then pathway enrichment analysis was conducted.
eFigure 8. A Heatmap Showing the Significantly More Inherited NKD Genes in TIME-Intermediate/TIME-Poor Subtypes Than TIME-Rich Subtype in Cancers

The digital numbers represent FDR-corrected p values. Columns and rows represent cancer types and known inherited NKD (NK cell deficiency) genes, respectively.
eFigure 9. A Bar Chart Showing Ratios of Gene Categories of the Potential NKD Genes Across 12 Cancer Types
**eFigure 10. Kaplan-Meier Curves of the High- and Low-Number of Functionally Inherited NK Cell Variants**

Kaplan-Meier curves of the patient groups of the high- and low-number of functionally inherited variants in the NK-defective genes for disease-free survival in cancers. Patients were top-ranked based on the number of functionally inherited variants in NK cells. Top 30% and Bottom 30% of the ranked patients were defined as the high- and low-number of the NK-defective patient groups, respectively.
**eFigure 11.** Negative Correlations Between the Number of the Inheritable Defective Genes and Abundance of TILs

NK, NKT, A CD8+ T, A CD4+ T, cDC, immature B, Activated B, MDSC, GD T, RT, CM CD8+ T, I dendritic, Type 1 TH, Type 2 TH, Type 17 TH, TF helper, A dendritic, EM CD4+ T, EM CD8+ T, P dendritic and CM CD4+ T represent natural killer cell, natural killer T cell, activated CD8+ T cell, activated CD4+ T cell, conventional dendritic cell, immature B cell, activated B cell, myeloid-derived suppressor cell, gamma delta T cell, regulatory T cell, central memory CD8+ T cell, immature dendritic cell, Type 1 T helper cell, Type 2 T helper cell, Type 17 T helper cell, T follicular helper cell, activated dendritic cell, effector memory CD4+ T cell, effector memory CD8+ T cell, plasmacytoid dendritic cell and central memory CD4+ T cell, respectively. *0.05<p-value <0.1; **0.01<p-value <0.05; and ***p-value<0.01.
**eFigure 12.** Kaplan-Meier Curves of the High- and Low-Number of Functionally Inherited Variants of the Combined Genes

Kaplan-Meier curves of the patient groups of the high- and low-number of merged functionally inherited variants in the combined genes (NK-defective genes + defective non-NK-specific ITAM-signaling genes) for disease-free survival in cancers. Patients were top-ranked based on the number of the functionally inherited variants. Top 30% and Bottom 30% of the ranked patients were defined as the high- and low-number of the defective patient groups, respectively.
eFigure 13. Negative Correlations Between the Number of the Inheritable Defective Combined Genes and Abundance of TILs

Negative correlations between the number of the inheritable defective genes of the combined genes (NK-defective genes + defective non-NK-specific ITAM-signaling genes) and the abundance of tumor-infiltrating lymphocytes in cancers. NK, NKT, A CD8+ T, A CD4+ T, cDC, immature B, Activated B, MDSC, GD T, RT, CM CD8+ T, I dendritic, Type 1 TH, Type 2 TH, Type 17 TH, TF helper, A dendritic, EM CD4+ T, EM CD8+ T, P dendritic and CM CD4+ T represent natural killer cell, natural killer T cell, activated CD8+ T cell, activated CD4+ T cell, conventional dendritic cell, immature B cell, activated B cell, myeloid-derived suppressor cell, gamma delta T cell, regulatory T cell, central memory CD8+ T cell, immature dendritic cell, Type 1 T helper cell, Type 2 T helper cell, Type 17 T helper cell, T follicular helper cell, activated dendritic cell, effector memory CD4+ T cell, effector memory CD8+ T cell, plasmacytoid dendritic cell and central memory CD4+ T cell, respectively. *0.05<p-value <0.1; **0.01<p-value <0.05; and ***p-value<0.01.
eFigure 14. The Abundance of TIL-NK Cells in the Tumors Bearing a Defective Gene in NK Cells Was Significantly Lower Than the Rest of the Tumors. T-tests were conducted.
**eFigure 15.** Heatmaps of the Significantly Differentially Functional Germline Variants Between Cancer and Cancer-Free Cohorts

Only the functional germline variants (rows) which are more enriched in cancer patients are shown. For each cancer types, the same number of the cancer-free individuals were randomly selected from the cancer-free cohort (n=4,500). Red and blue bars represent cancer and cancer-free individuals, respectively. Columns represent samples.
eFigure 16. Significantly Enriched Pathways Derived from the Significantly Differential Germline Variants Between Individuals With No Cancer Patients With Cancer

A heatmap showing the significantly enriched pathways derived from the significantly differential germline variants between cancer-free individuals and cancer patients in 13 cancer types. Columns and rows represent KEGG pathways and cancer types, respectively. The digital numbers represent FDR-corrected p values. Significantly functional differential germline variants (p<0.2) between cancer-free individuals and cancer patients were intersected with the immune-checkpoint therapy (ICT) essential genes, and then pathway enrichment analysis was conducted.
**eFigure 17. Kaplan-Meier Curves of the High- and Low-Number of Functionally Inherited Variants in the Wnt Signaling Pathway for Disease-Free Survival**

Patients were top-to-bottom ranked based on the number of functionally inherited variants in the WNT signaling pathway. Top 30% and Bottom 30% of the ranked patients were defined as the high- and low-number of the defected defects in the pathway, respectively.
**eFigure 18.** Correlations of the Functionally Inherited Variants in the Wnt Signaling Pathway With the Abundance of TILs

Bar charts illustrating the number of the functionally inherited variants (inherited defects) in the Wnt signaling pathway is negatively correlated with the abundances of tumor-infiltrating lymphocytes. Cancer types: head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), lower grade glioma (LGG), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC) and skin cutaneous melanoma (SKCM). * p<0.05; ** 0.05<p<0.001 and *** p<0.001.
**eTable 1.** List of ITAM-Signaling Genes

| ITAM-signaling genes |
|----------------------|
| ULBP1                |
| ULBP2                |
| ULBP3                |
| MICA                 |
| MICB                 |
| CD94                 |
| NKG2A                |
| NKG2C                |
| HLA-E                |
| CD16                 |
| DNAM-1               |
| CD226                |
| ITGB2                |
| ICAM1                |
| ZAP70                |
| PLCG1                |
| VAV1                 |
| PLCG2                |
**eTable 2.** List of Ligands of the NK Activating Receptors

| Ligands of NK cell activating receptors |
|----------------------------------------|
| TNFRSF14                               |
| PVR                                    |
| NECTIN2                                |
| CD48                                   |
| CADM1                                  |
| HLA-E                                  |
| MICA                                   |
| MICB                                   |
| RAET1E                                 |
| RAET1G                                 |
| RAET1F                                 |
| ULBP1                                  |
| ULBP2                                  |
| ULBP3                                  |
| RAET1L                                 |
| VIM                                    |
| KMT2E                                  |
| PCNA                                   |
| PDGFD                                  |
| BAG6                                   |
| NCR3LG1                                |
| TNFSF9                                 |
| CD274                                  |
| PDCD1LG2                               |
| FAS                                    |
| FASLG                                  |
| Protein   |
|-----------|
| TNFRSF10B |
| TNFRSF10A |
| ICAM1     |
| CSF2      |
| CSF1      |
| CSF3      |
| CD70      |
| SELL      |
| CD72      |
| CLEC2B    |
| CEACAM1   |
| SLAMF6    |
| SLAMF7    |
### eTable 3. Average Immune Cell Fractions for TIME-Rich, TIME-Intermediate and TIME-Poor Subtypes, Respectively

| Cancer | Immune cell                      | TIME-rich | TIME-intermediate | TIME-poor | P-value   |
|--------|----------------------------------|-----------|-------------------|-----------|-----------|
| BLCA   | Activated CD4+ T cell            | 0.25      | 0.18              | 0.14      | 1.22E-19  |
| BLCA   | Activated CD8+ T cell            | 0.26      | 0.14              | 0.14      | 9.75E-19  |
| BLCA   | Activated dendritic cell         | 0.26      | 0.21              | 0.17      | 6.37E-27  |
| BLCA   | CD56<sup>bright</sup> natural killer cell | 0.27  | 0.25              | 0.25      | 7.75E-09  |
| BLCA   | CD56<sup>dim</sup> natural killer cell | 0.33 | 0.33              | 0.34      | 4.73E-01  |
| BLCA   | Central memory CD4+ T cell       | 0.55      | 0.52              | 0.50      | 2.24E-13  |
| BLCA   | Central memory CD8+ T cell       | 0.35      | 0.33              | 0.30      | 5.10E-08  |
| BLCA   | Effector memory CD4+ T cell      | 0.16      | 0.15              | 0.13      | 3.12E-03  |
| BLCA   | Effector memory CD8+ T cell      | 0.32      | 0.20              | 0.15      | 4.70E-31  |
| BLCA   | Gamma delta T cell               | 0.29      | 0.25              | 0.24      | 4.65E-16  |
| BLCA   | Immature dendritic cell          | 0.35      | 0.34              | 0.33      | 5.46E-04  |
| BLCA   | MDSC                             | 0.36      | 0.20              | 0.13      | 2.30E-20  |
| BLCA   | Memory B cell                    | 0.10      | 0.09              | 0.07      | 3.07E-03  |
| BLCA   | Monocyte                         | 0.36      | 0.36              | 0.36      | 6.73E-01  |
| BLCA   | Natural killer cell              | 0.28      | 0.24              | 0.20      | 7.85E-17  |
| BLCA   | Plasmacytoid dendritic cell      | 0.38      | 0.36              | 0.34      | 3.59E-07  |
| Tissue | Cell Type                        | Value1 | Value2 | Value3 | Value4 |
|--------|----------------------------------|--------|--------|--------|--------|
| BLCA   | T follicular helper cell         | 0.18   | 0.13   | 0.10   | 5.61E-14 |
| BLCA   | Type 1 T helper cell             | 0.17   | 0.12   | 0.09   | 1.53E-16 |
| BLCA   | Type 2 T helper cell             | 0.15   | 0.13   | 0.10   | 3.66E-12 |
| BRCA   | Activated CD4+ T cell            | 0.20   | 0.14   | 0.10   | 5.66E-09 |
| BRCA   | Activated CD8+ T cell            | 0.24   | 0.14   | 0.12   | 3.11E-13 |
| BRCA   | Activated dendritic cell         | 0.25   | 0.20   | 0.20   | 9.72E-15 |
| BRCA   | CD56° bright natural killer cell | 0.25   | 0.22   | 0.22   | 1.23E-13 |
| BRCA   | CD56° dim natural killer cell    | 0.35   | 0.33   | 0.32   | 7.54E-04 |
| BRCA   | Central memory CD4+ T cell       | 0.54   | 0.51   | 0.51   | 6.63E-08 |
| BRCA   | Central memory CD8+ T cell       | 0.35   | 0.34   | 0.35   | 2.56E-01 |
| BRCA   | Effector memory CD4+ T cell      | 0.16   | 0.15   | 0.15   | 3.61E-02 |
| BRCA   | Effector memory CD8+ T cell      | 0.29   | 0.20   | 0.20   | 4.10E-11 |
| BRCA   | Gamma delta T cell               | 0.25   | 0.23   | 0.22   | 1.55E-07 |
| BRCA   | Immature dendritic cell          | 0.34   | 0.33   | 0.34   | 1.58E-01 |
| BRCA   | MDSC                             | 0.34   | 0.22   | 0.20   | 7.81E-13 |
| BRCA   | Memory B cell                    | 0.13   | 0.14   | 0.13   | 7.10E-01 |
| BRCA   | Monocyte                         | 0.36   | 0.33   | 0.33   | 4.11E-10 |
| BRCA   | Natural killer cell              | 0.29   | 0.26   | 0.27   | 2.65E-04 |
| BRCA   | Natural killer T cell            | 0.09   | 0.05   | 0.05   | 4.08E-10 |
| BRCA   | Plasmacytoid dendritic cell      | 0.40   | 0.38   | 0.39   | 4.96E-04 |
| Cancer Type | Cell Type                        | Value1 | Value2 | Value3 | Value4 |
|-------------|----------------------------------|--------|--------|--------|--------|
| BRCA        | Regulatory T cell                | 0.20   | 0.12   | 0.12   | 1.77E-09 |
| BRCA        | T follicular helper cell         | 0.16   | 0.12   | 0.11   | 5.60E-09 |
| BRCA        | Type 1 T helper cell             | 0.17   | 0.12   | 0.12   | 1.19E-09 |
| BRCA        | Type 2 T helper cell             | 0.16   | 0.13   | 0.13   | 3.51E-03 |
| COAD        | Activated CD4+ T cell            | 0.10   | 0.21   | 0.19   | 8.52E-03 |
| COAD        | Activated CD8+ T cell            | 0.19   | 0.21   | 0.17   | 1.61E-01 |
| COAD        | Activated dendritic cell         | 0.27   | 0.28   | 0.25   | 6.01E-02 |
| COAD        | CD56^bright^ natural killer cell | 0.26   | 0.25   | 0.25   | 3.90E-02 |
| COAD        | CD56^dim^ natural killer cell    | 0.45   | 0.40   | 0.39   | 1.31E-04 |
| COAD        | Central memory CD4+ T cell       | 0.55   | 0.54   | 0.55   | 6.76E-01 |
| COAD        | Central memory CD8+ T cell       | 0.34   | 0.34   | 0.34   | 8.83E-01 |
| COAD        | Effector memory CD4+ T cell      | 0.06   | 0.10   | 0.12   | 4.12E-03 |
| COAD        | Effector memory CD8+ T cell      | 0.18   | 0.21   | 0.17   | 5.47E-01 |
| COAD        | Gamma delta T cell               | 0.29   | 0.28   | 0.28   | 2.15E-01 |
| COAD        | Immature dendritic cell          | 0.27   | 0.29   | 0.30   | 8.29E-02 |
| COAD        | MDSC                             | 0.24   | 0.22   | 0.19   | 6.22E-02 |
| COAD        | Memory B cell                    | 0.13   | 0.14   | 0.15   | 1.62E-04 |
| COAD        | Monocyte                         | 0.41   | 0.40   | 0.39   | 4.50E-02 |
| COAD        | Natural killer cell              | 0.27   | 0.27   | 0.27   | 7.70E-01 |
| COAD        | Natural killer T cell            | 0.06   | 0.05   | 0.05   | 1.41E-01 |
| Tumor Type | Cell Type                        | Value 1 | Value 2 | Value 3 | Value 4       |
|------------|---------------------------------|---------|---------|---------|--------------|
| COAD       | Plasmacytoid dendritic cell     | 0.37    | 0.37    | 0.38    | 5.93E-01     |
| COAD       | Regulatory T cell               | 0.14    | 0.14    | 0.15    | 6.78E-01     |
| COAD       | T follicular helper cell        | 0.13    | 0.12    | 0.11    | 1.70E-01     |
| COAD       | Type 1 T helper cell            | 0.15    | 0.14    | 0.13    | 1.78E-01     |
| COAD       | Type 2 T helper cell            | 0.03    | 0.08    | 0.07    | 3.72E-02     |
| HNSC       | Activated CD4+ T cell           | 0.22    | 0.20    | 0.19    | 2.47E-03     |
| HNSC       | Activated CD8+ T cell           | 0.25    | 0.17    | 0.15    | 3.13E-19     |
| HNSC       | Activated dendritic cell        | 0.28    | 0.25    | 0.23    | 3.09E-15     |
| HNSC       | CD56bright natural killer cell  | 0.31    | 0.30    | 0.29    | 5.75E-10     |
| HNSC       | CD56dim natural killer cell     | 0.36    | 0.36    | 0.35    | 4.98E-01     |
| HNSC       | Central memory CD4+ T cell      | 0.59    | 0.58    | 0.56    | 4.85E-10     |
| HNSC       | Central memory CD8+ T cell      | 0.37    | 0.37    | 0.36    | 4.95E-02     |
| HNSC       | Effector memory CD4+ T cell     | 0.19    | 0.20    | 0.19    | 8.72E-01     |
| HNSC       | Effector memory CD8+ T cell     | 0.33    | 0.29    | 0.25    | 1.01E-17     |
| HNSC       | Gamma delta T cell              | 0.31    | 0.29    | 0.27    | 1.16E-09     |
| HNSC       | Immature dendritic cell         | 0.38    | 0.39    | 0.38    | 3.40E-01     |
| HNSC       | MDSC                            | 0.37    | 0.29    | 0.24    | 2.11E-17     |
| HNSC       | Memory B cell                   | 0.13    | 0.12    | 0.12    | 2.92E-01     |
| HNSC       | Monocyte                        | 0.37    | 0.36    | 0.35    | 2.28E-09     |
| HNSC       | Natural killer cell             | 0.28    | 0.26    | 0.25    | 1.69E-05     |
| Tumor Type | Cell Type                          | Count HNSC | Count KIRC | Count KIRC | Count HNSC | Count KIRC | Count KIRC | Count HNSC | Count KIRC | Count KIRC | Count HNSC | Count KIRC | Count KIRC | Count HNSC | Count KIRC | Count KIRC | Count KIRC | Count HNSC | Count KIRC | Count KIRC | Count HNSC | Count KIRC | Count KIRC |
|------------|-----------------------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| HNSC       | Natural killer T cell             | 0.13       | 0.11       | 0.09       | 9.38E-12   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| HNSC       | Plasmacytoid dendritic cell       | 0.42       | 0.42       | 0.41       | 1.81E-01   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| HNSC       | Regulatory T cell                 | 0.23       | 0.18       | 0.13       | 5.87E-11   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| HNSC       | T follicular helper cell          | 0.19       | 0.17       | 0.15       | 9.16E-08   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| HNSC       | Type 1 T helper cell              | 0.18       | 0.16       | 0.13       | 1.55E-11   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| HNSC       | Type 2 T helper cell              | 0.16       | 0.17       | 0.15       | 2.86E-01   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | Activated CD8+ T cell             | 0.30       | 0.22       | 0.13       | 1.22E-17   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | Activated dendritic cell          | 0.32       | 0.29       | 0.25       | 7.56E-20   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | CD56<sup>bright</sup> natural killer cell | 0.25   | 0.24       | 0.22       | 1.62E-16   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | CD56<sup>dim</sup> natural killer cell | 0.40   | 0.40       | 0.39       | 2.05E-01   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | Central memory CD4+ T cell        | 0.60       | 0.59       | 0.56       | 4.58E-10   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | Central memory CD8+ T cell        | 0.37       | 0.37       | 0.34       | 9.71E-03   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | Effector memory CD4+ T cell       | 0.25       | 0.26       | 0.24       | 1.31E-01   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | Effector memory CD8+ T cell       | 0.36       | 0.33       | 0.30       | 3.14E-10   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | Gamma delta T cell                | 0.31       | 0.30       | 0.27       | 9.25E-10   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | Immature dendritic cell           | 0.39       | 0.39       | 0.38       | 1.10E-02   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | MDSC                              | 0.43       | 0.37       | 0.28       | 3.02E-20   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | Memory B cell                     | 0.13       | 0.12       | 0.12       | 5.34E-03   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | Monocyte                          | 0.42       | 0.41       | 0.40       | 3.23E-06   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
| KIRC       | Natural killer cell               | 0.38       | 0.38       | 0.36       | 2.54E-04   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |

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| Tumor Type | Cell Type                        | Mean   | Median | StdDev | p-Value   |
|------------|----------------------------------|--------|--------|--------|-----------|
| KIRC       | Natural killer T cell            | 0.16   | 0.15   | 0.13   | 3.33E-06  |
| KIRC       | Plasmacytoid dendritic cell      | 0.45   | 0.45   | 0.45   | 3.12E-01  |
| KIRC       | Regulatory T cell                | 0.28   | 0.26   | 0.22   | 1.44E-06  |
| KIRC       | T follicular helper cell         | 0.25   | 0.23   | 0.20   | 2.29E-13  |
| KIRC       | Type 1 T helper cell             | 0.24   | 0.22   | 0.19   | 1.41E-14  |
| KIRC       | Type 2 T helper cell             | 0.12   | 0.11   | 0.10   | 1.01E-01  |
| LGG        | Activated dendritic cell         | 0.21   | 0.16   | 0.15   | 6.58E-28  |
| LGG        | CD56bright natural killer cell   | 0.24   | 0.26   | 0.24   | 1.86E-03  |
| LGG        | CD56dim natural killer cell      | 0.34   | 0.31   | 0.32   | 9.05E-08  |
| LGG        | Central memory CD4++ T cell      | 0.48   | 0.44   | 0.43   | 1.29E-22  |
| LGG        | Central memory CD8+ T cell       | 0.24   | 0.21   | 0.20   | 1.52E-12  |
| LGG        | Effector memory CD4+ T cell      | 0.26   | 0.24   | 0.27   | 5.80E-03  |
| LGG        | Gamma delta T cell               | 0.16   | 0.13   | 0.12   | 6.87E-15  |
| LGG        | Immature dendritic cell          | 0.35   | 0.32   | 0.33   | 2.07E-24  |
| LGG        | Monocyte                         | 0.43   | 0.43   | 0.43   | 3.39E-02  |
| LGG        | Natural killer cell              | 0.28   | 0.22   | 0.22   | 1.13E-31  |
| LGG        | Plasmacytoid dendritic cell      | 0.41   | 0.38   | 0.38   | 5.44E-30  |
| LGG        | T follicular helper cell         | 0.23   | 0.19   | 0.19   | 3.70E-19  |
| LGG        | Type 1 T helper cell             | 0.12   | 0.06   | 0.07   | 1.61E-42  |
| LGG        | Type 2 T helper cell             | 0.12   | 0.11   | 0.11   | 2.75E-04  |

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| LUAD                  | Activated CD4+ T cell | 0.14 | 0.19 | 0.16 | 8.58E-04 |
|-----------------------|-----------------------|------|------|------|----------|
| LUAD                  | Activated CD8+ T cell | 0.20 | 0.22 | 0.16 | 9.64E-01 |
| LUAD                  | Activated dendritic cell | 0.30 | 0.30 | 0.25 | 3.16E-05 |
| LUAD                  | CD56\textsuperscript{Bright} natural killer cell | 0.25 | 0.25 | 0.23 | 1.64E-02 |
| LUAD                  | CD56\textsuperscript{dim} natural killer cell | 0.38 | 0.39 | 0.38 | 2.21E-01 |
| LUAD                  | Central memory CD4+ T cell | 0.60 | 0.60 | 0.57 | 1.50E-04 |
| LUAD                  | Central memory CD8+ T cell | 0.38 | 0.37 | 0.33 | 7.79E-04 |
| LUAD                  | Effector memory CD4+ T cell | 0.18 | 0.17 | 0.16 | 6.38E-03 |
| LUAD                  | Effector memory CD8+ T cell | 0.34 | 0.33 | 0.24 | 1.67E-06 |
| LUAD                  | Gamma delta T cell | 0.30 | 0.29 | 0.26 | 8.24E-03 |
| LUAD                  | Immature dendritic cell | 0.42 | 0.40 | 0.38 | 4.80E-09 |
| LUAD                  | MDSC | 0.40 | 0.39 | 0.28 | 3.57E-05 |
| LUAD                  | Monocyte | 0.41 | 0.40 | 0.38 | 1.20E-05 |
| LUAD                  | Natural killer cell | 0.31 | 0.31 | 0.27 | 9.23E-05 |
| LUAD                  | Natural killer T cell | 0.10 | 0.11 | 0.07 | 1.13E-01 |
| LUAD                  | Plasmacytoid dendritic cell | 0.42 | 0.41 | 0.38 | 2.99E-05 |
| LUAD                  | Regulatory T cell | 0.30 | 0.28 | 0.19 | 5.39E-05 |
| LUAD                  | T follicular helper cell | 0.22 | 0.20 | 0.16 | 5.67E-11 |
| LUAD                  | Type 1 T helper cell | 0.19 | 0.19 | 0.14 | 2.03E-03 |
| LUAD                  | Type 2 T helper cell | 0.15 | 0.15 | 0.15 | 5.81E-01 |

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|                | Count 1 | Count 2 | Count 3 | p-value       |
|----------------|--------|--------|--------|--------------|
| LUSC Activated CD4+ T cell | 0.25   | 0.20   | 0.21   | 3.37E-12     |
| LUSC Activated CD8+ T cell | 0.22   | 0.14   | 0.17   | 1.88E-18     |
| LUSC Activated dendritic cell | 0.28   | 0.22   | 0.23   | 3.80E-45     |
| LUSC CD56<sup>+</sup> natural killer cell | 0.27   | 0.27   | 0.28   | 5.93E-02     |
| LUSC CD56<sup>+</sup> natural killer cell | 0.35   | 0.32   | 0.34   | 2.50E-09     |
| LUSC Central memory CD4+ T cell | 0.56   | 0.52   | 0.52   | 1.92E-30     |
| LUSC Central memory CD8+ T cell | 0.37   | 0.33   | 0.34   | 4.43E-16     |
| LUSC Effector memory CD4+ T cell | 0.19   | 0.17   | 0.17   | 5.81E-11     |
| LUSC Effector memory CD8+ T cell | 0.32   | 0.21   | 0.22   | 9.75E-44     |
| LUSC Gamma delta T cell | 0.29   | 0.24   | 0.25   | 3.61E-28     |
| LUSC Immature dendritic cell | 0.38   | 0.36   | 0.36   | 2.00E-11     |
| LUSC MDSC | 0.39   | 0.24   | 0.25   | 4.27E-40     |
| LUSC Memory B cell | 0.11   | 0.11   | 0.11   | 4.94E-01     |
| LUSC Monocyte | 0.36   | 0.33   | 0.33   | 1.46E-19     |
| LUSC Natural killer cell | 0.28   | 0.23   | 0.23   | 6.95E-33     |
| LUSC Natural killer T cell | 0.13   | 0.08   | 0.09   | 2.89E-26     |
| LUSC Plasmacytoid dendritic cell | 0.40   | 0.37   | 0.38   | 5.80E-19     |
| LUSC Regulatory T cell | 0.29   | 0.15   | 0.17   | 1.62E-38     |
| LUSC T follicular helper cell | 0.19   | 0.13   | 0.14   | 4.45E-36     |
| LUSC Type 1 T helper cell | 0.19   | 0.12   | 0.13   | 1.63E-34     |

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| Tumor Type | Cell Type                        | Mean 1 | Mean 2 | Mean 3 | p-value     |
|------------|----------------------------------|--------|--------|--------|-------------|
| LUSC       | Type 2 T helper cell             | 0.16   | 0.13   | 0.14   | 4.46E-11    |
| PRAD       | Activated CD8+ T cell            | 0.16   | 0.17   | 0.14   | 7.22E-01    |
| PRAD       | Activated dendritic cell         | 0.19   | 0.22   | 0.20   | 2.22E-06    |
| PRAD       | CD56^bright natural killer cell  | 0.30   | 0.31   | 0.30   | 2.17E-02    |
| PRAD       | CD56^dim natural killer cell     | 0.30   | 0.31   | 0.30   | 2.62E-03    |
| PRAD       | Central memory CD4+ T cell       | 0.50   | 0.53   | 0.51   | 4.28E-07    |
| PRAD       | Central memory CD8+ T cell       | 0.23   | 0.24   | 0.24   | 2.55E-06    |
| PRAD       | Effector memory CD4+ T cell      | 0.14   | 0.16   | 0.16   | 5.23E-08    |
| PRAD       | Effector memory CD8+ T cell      | 0.15   | 0.19   | 0.16   | 2.26E-06    |
| PRAD       | Gamma delta T cell               | 0.17   | 0.18   | 0.17   | 2.34E-01    |
| PRAD       | Immature dendritic cell          | 0.35   | 0.36   | 0.36   | 7.58E-07    |
| PRAD       | Memory B cell                    | 0.05   | 0.05   | 0.06   | 1.09E-02    |
| PRAD       | Monocyte                         | 0.43   | 0.42   | 0.42   | 2.02E-01    |
| PRAD       | Natural killer cell              | 0.25   | 0.28   | 0.27   | 1.22E-14    |
| PRAD       | Plasmacytoid dendritic cell      | 0.40   | 0.41   | 0.41   | 4.11E-07    |
| PRAD       | T follicular helper cell         | 0.11   | 0.14   | 0.13   | 4.61E-10    |
| PRAD       | Type 1 T helper cell             | 0.06   | 0.09   | 0.07   | 2.79E-08    |
| SKCM       | Activated CD4+ T cell            | 0.24   | 0.21   | 0.14   | 2.44E-13    |
| SKCM       | Activated CD8+ T cell            | 0.28   | 0.16   | 0.12   | 2.51E-24    |
| Dataset | Cell Type                          | Mean1 | Mean2 | Mean3 | P-Value   |
|---------|-----------------------------------|-------|-------|-------|-----------|
| SKCM    | Activated dendritic cell          | 0.28  | 0.23  | 0.19  | 1.45E-32  |
| SKCM    | CD56<sup>bright</sup> natural killer cell | 0.25  | 0.23  | 0.24  | 2.32E-09  |
| SKCM    | CD56<sup>dim</sup> natural killer cell | 0.34  | 0.34  | 0.33  | 2.05E-02  |
| SKCM    | Central memory CD4+ T cell        | 0.54  | 0.51  | 0.48  | 1.63E-22  |
| SKCM    | Central memory CD8+ T cell        | 0.34  | 0.33  | 0.30  | 1.33E-09  |
| SKCM    | Effector memory CD4+ T cell       | 0.17  | 0.17  | 0.13  | 1.30E-05  |
| SKCM    | Effector memory CD8+ T cell       | 0.34  | 0.24  | 0.19  | 4.02E-33  |
| SKCM    | Gamma delta T cell                | 0.29  | 0.28  | 0.26  | 5.88E-09  |
| SKCM    | Immature dendritic cell           | 0.36  | 0.35  | 0.32  | 5.15E-12  |
| SKCM    | MDSC                              | 0.38  | 0.26  | 0.19  | 9.97E-26  |
| SKCM    | Memory B cell                     | 0.18  | 0.19  | 0.17  | 3.63E-01  |
| SKCM    | Monocyte                          | 0.42  | 0.40  | 0.39  | 1.96E-15  |
| SKCM    | Natural killer cell               | 0.32  | 0.28  | 0.25  | 2.93E-24  |
| SKCM    | Natural killer T cell             | 0.13  | 0.10  | 0.07  | 1.57E-18  |
| SKCM    | Plasmacytoid dendritic cell       | 0.42  | 0.41  | 0.40  | 4.31E-09  |
| SKCM    | Regulatory T cell                 | 0.27  | 0.20  | 0.14  | 5.36E-19  |
| SKCM    | T follicular helper cell          | 0.23  | 0.18  | 0.14  | 1.79E-24  |
| SKCM    | Type 1 T helper cell              | 0.19  | 0.15  | 0.11  | 1.49E-22  |
| SKCM    | Type 2 T helper cell              | 0.18  | 0.19  | 0.17  | 6.74E-01  |
| STAD    | Activated CD4+ T cell             | 0.24  | 0.23  | 0.28  | 2.27E-08  |
| STAD         | Cell Type                          | STAD Value | STAD Value | STAD Value | STAD Value |
|-------------|------------------------------------|------------|------------|------------|------------|
| STAD        | Activated CD8+ T cell              | 0.08       | 0.16       | 0.27       | 2.95E-24   |
| STAD        | Activated dendritic cell           | 0.19       | 0.25       | 0.31       | 1.76E-28   |
| STAD        | CD56<sup>+</sup> natural killer cell | 0.18       | 0.18       | 0.26       | 8.35E-06   |
| STAD        | CD56<sup>-</sup> natural killer cell | 0.39       | 0.38       | 0.45       | 1.88E-01   |
| STAD        | Central memory CD4+ T cell         | 0.51       | 0.55       | 0.57       | 3.60E-24   |
| STAD        | Central memory CD8+ T cell         | 0.29       | 0.33       | 0.42       | 6.85E-16   |
| STAD        | Effector memory CD4+ T cell        | 0.16       | 0.21       | 0.13       | 7.31E-10   |
| STAD        | Effector memory CD8+ T cell        | 0.13       | 0.25       | 0.30       | 2.27E-41   |
| STAD        | Gamma delta T cell                 | 0.19       | 0.21       | 0.26       | 2.05E-08   |
| STAD        | Immature dendritic cell            | 0.31       | 0.27       | 0.26       | 2.31E-03   |
| STAD        | MDSC                               | 0.08       | 0.20       | 0.39       | 1.54E-33   |
| STAD        | Memory B cell                      | 0.15       | 0.12       | 0.08       | 7.92E-04   |
| STAD        | Monocyte                           | 0.32       | 0.40       | 0.42       | 1.02E-17   |
| STAD        | Natural killer cell                | 0.29       | 0.31       | 0.34       | 8.21E-27   |
| STAD        | Natural killer T cell              | 0.09       | 0.10       | 0.09       | 8.02E-23   |
| STAD        | Plasmacytoid dendritic cell        | 0.39       | 0.42       | 0.38       | 7.05E-07   |
| STAD        | Regulatory T cell                  | 0.02       | 0.10       | 0.19       | 1.05E-21   |
| STAD        | T follicular helper cell           | 0.07       | 0.15       | 0.20       | 4.73E-24   |
| STAD        | Type 1 T helper cell               | 0.09       | 0.18       | 0.19       | 6.06E-32   |
| STAD        | Type 2 T helper cell               | 0.18       | 0.14       | 0.09       | 1.20E-07   |
|                |                          |         |         |               |
|----------------|--------------------------|---------|---------|---------------|
| THCA Activated CD8+ T cell | 0.19 | 0.09 | 0.06 | 8.27E-34 |
| THCA Activated dendritic cell | 0.26 | 0.21 | 0.17 | 4.08E-45 |
| THCA CD56<sup>**high**</sup> natural killer cell | 0.26 | 0.23 | 0.22 | 2.18E-51 |
| THCA CD56<sup>**dim**</sup> natural killer cell | 0.38 | 0.37 | 0.32 | 3.02E-26 |
| THCA Central memory CD4+ T cell | 0.58 | 0.55 | 0.50 | 1.07E-45 |
| THCA Central memory CD8+ T cell | 0.36 | 0.32 | 0.30 | 6.76E-28 |
| THCA Effector memory CD4+ T cell | 0.19 | 0.18 | 0.17 | 1.59E-09 |
| THCA Effector memory CD8+ T cell | 0.29 | 0.22 | 0.12 | 1.42E-44 |
| THCA Gamma delta T cell | 0.25 | 0.22 | 0.18 | 1.72E-33 |
| THCA Immature dendritic cell | 0.41 | 0.39 | 0.34 | 1.85E-28 |
| THCA Monocyte | 0.44 | 0.42 | 0.42 | 7.49E-24 |
| THCA Natural killer cell | 0.34 | 0.33 | 0.28 | 2.07E-20 |
| THCA Plasmacytoid dendritic cell | 0.41 | 0.40 | 0.39 | 1.80E-21 |
| THCA T follicular helper cell | 0.24 | 0.21 | 0.17 | 5.73E-30 |
| THCA Type 1 T helper cell | 0.18 | 0.14 | 0.11 | 4.66E-33 |
| THCA Type 2 T helper cell | 0.07 | 0.06 | 0.04 | 2.26E-05 |
| UCEC Activated CD8+ T cell | 0.20 | 0.17 | 0.14 | 2.71E-02 |
| UCEC Activated dendritic cell | 0.25 | 0.22 | 0.19 | 1.66E-05 |
| UCEC                  | CD56bright natural killer cell | 0.25 | 0.25 | 0.23 | 3.46E-04 |
|----------------------|--------------------------------|------|------|------|----------|
| UCEC                  | CD56dim natural killer cell    | 0.38 | 0.33 | 0.33 | 1.61E-07 |
| UCEC                  | Central memory CD4+ T cell     | 0.49 | 0.49 | 0.47 | 6.55E-02 |
| UCEC                  | Central memory CD8+ T cell     | 0.24 | 0.23 | 0.22 | 1.54E-02 |
| UCEC                  | Effector memory CD4+ T cell    | 0.05 | 0.09 | 0.08 | 1.34E-03 |
| UCEC                  | Effector memory CD8+ T cell    | 0.21 | 0.19 | 0.14 | 2.98E-03 |
| UCEC                  | Gamma delta T cell             | 0.21 | 0.22 | 0.21 | 7.41E-01 |
| UCEC                  | Immature dendritic cell        | 0.30 | 0.32 | 0.30 | 2.00E-01 |
| UCEC                  | MDSC                           | 0.28 | 0.20 | 0.15 | 5.91E-05 |
| UCEC                  | Memory B cell                  | 0.08 | 0.06 | 0.07 | 1.42E-01 |
| UCEC                  | Monocyte                       | 0.36 | 0.35 | 0.34 | 1.19E-03 |
| UCEC                  | Natural killer cell            | 0.26 | 0.24 | 0.23 | 5.15E-04 |
| UCEC                  | Plasmacytoid dendritic cell    | 0.35 | 0.36 | 0.35 | 4.81E-01 |
| UCEC                  | T follicular helper cell        | 0.14 | 0.10 | 0.09 | 2.29E-05 |
| UCEC                  | Type 1 T helper cell           | 0.12 | 0.10 | 0.08 | 5.12E-04 |
| UCEC                  | Type 2 T helper cell           | 0.08 | 0.09 | 0.09 | 2.29E-02 |

Note: p-values represent the significant differences for each type of immune cells between the TIME-rich and TIME-intermediate/-poor subtypes.
eTable 4. Fractions of the Patients in TIME-Rich, TIME-Intermediate, and TIME-Poor Subtype in Cancers

| Cancer  | Patient (n) | TIME-rich (%) | TIME-intermediate (%) | TIME-poor (%) |
|---------|-------------|---------------|-----------------------|---------------|
| UCEC    | 365         | 7.5           | 20.3                  | 72.3          |
| LUAD    | 512         | 17.4          | 52.5                  | 30.1          |
| BLCA    | 408         | 19.4          | 51.5                  | 29.2          |
| BRCA    | 314         | 22.0          | 37.2                  | 40.8          |
| STAD    | 374         | 22.2          | 11.5                  | 66.3          |
| KIRC    | 530         | 23.8          | 38.7                  | 37.5          |
| PRAD    | 497         | 26.4          | 13.1                  | 60.6          |
| COAD    | 87          | 26.4          | 16.1                  | 57.5          |
| HNSC    | 412         | 28.6          | 39.3                  | 32.0          |
| THCA    | 498         | 29.3          | 44.8                  | 25.9          |
| LUSC    | 501         | 30.7          | 40.1                  | 29.1          |
| SKCM    | 364         | 34.3          | 29.1                  | 36.5          |
| LGG     | 511         | 41.7          | 34.1                  | 24.3          |
**eTable 5. NK Defective Genes in Each Cancer Type**

| Cancer type | Gene       | Annotation                  |
|-------------|------------|-----------------------------|
| LGG         | CMA1       | NK cell                     |
|             | CSF2       | NK cell+NKT cell+γδ T cell   |
|             | FASLG      | NK cell+NKT cell             |
|             | IKZF3      | NK cell+NKT cell             |
|             | KIR2DL4    | NK cell+NKT cell             |
|             | KIR3DL3    | NK cell+NKT cell             |
|             | KLHL30     | NK cell                     |
|             | NCR1       | NK cell+γδ T cell            |
|             | NCR2       | NK cell                     |
|             | NCR3       | NK cell                     |
|             | SH2D1B     | NK cell+γδ T cell            |
| BRCA        | C17orf66   | NK cell+NKT cell             |
|             | CD244      | NK cell+NKT cell+γδ T cell   |
|             | CSF2       | NK cell+NKT cell+γδ T cell   |
|             | KHDC1      | NK cell                     |
|             | KIR2DL3    | NK cell+NKT cell             |
|             | KIR2DL4    | NK cell+NKT cell             |
|             | KIR3DL2    | NK cell+NKT cell             |
|             | KIR3DL3    | NK cell+NKT cell             |
|             | KLHL30     | NK cell                     |
|             | KLRF1      | NK cell                     |
|             | NCR3       | NK cell                     |
|             | SH2D1B     | NK cell+γδ T cell            |
| HNSC        | C17orf66   | NK cell+NKT cell             |
|             | CD244      | NK cell+NKT cell+γδ T cell   |
|             | CDC20B     | NK cell+γδ T cell            |

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| Gene     | Expression Pattern          |
|----------|-----------------------------|
| CHRNE    | NK cell                     |
| CLNK     | NK cell + γδ T cell         |
| CMA1     | NK cell                     |
| KIR2DL3  | NK cell + NKT cell          |
| KIR2DL4  | NK cell + NKT cell          |
| KIR3DL1  | NK cell + NKT cell          |
| KIR3DL3  | NK cell + NKT cell          |
| KLRF1    | NK cell                     |
| NCR2     | NK cell                     |
| NCR3     | NK cell                     |
| C17orf66 | NK cell + NKT cell          |
| CDC20B   | NK cell + γδ T cell         |
| CLNK     | NK cell + γδ T cell         |
| CMA1     | NK cell                     |
| CSF2     | NK cell + NKT cell + γδ T cell |
| IKZF3    | NK cell + NKT cell          |
| KHDC1    | NK cell                     |
| KIR2DL1  | NK cell + NKT cell          |
| KIR2DL3  | NK cell + NKT cell          |
| KIR3DL1  | NK cell + NKT cell          |
| KIR3DL2  | NK cell + NKT cell          |
| KIR3DL3  | NK cell + NKT cell          |
| KLHL30   | NK cell                     |
| KLKB1    | NK cell + NKT cell + γδ T cell |
| NCR2     | NK cell                     |
| NCR3     | NK cell                     |
| SH2D1B   | NK cell + γδ T cell         |
| C17orf66 | NK cell + NKT cell          |
| CD244    | NK cell + NKT cell + γδ T cell |

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| Gene       | Description                  |
|------------|------------------------------|
| CDC20B     | NK cell + γδ T cell          |
| CHRNE      | NK cell                      |
| CLNK       | NK cell + γδ T cell          |
| CMA1       | NK cell                      |
| CSF2       | NK cell + NKT cell + γδ T cell|
| FASLG      | NK cell + NKT cell           |
| KIR2DL1    | NK cell + NKT cell           |
| KIR2DL3    | NK cell + NKT cell           |
| KIR3DL1    | NK cell + NKT cell           |
| KIR3DL2    | NK cell + NKT cell           |
| KLHL30     | NK cell                      |
| KLRF1      | NK cell                      |
| NCR2       | NK cell                      |
| SH2D1B     | NK cell + γδ T cell          |
| C17orf66   | NK cell + NKT cell           |
| CD244      | NK cell + NKT cell + γδ T cell|
| CDC20B     | NK cell + γδ T cell          |
| CMA1       | NK cell                      |
| CSF2       | NK cell + NKT cell + γδ T cell|
| FASLG      | NK cell + NKT cell           |
| KHDC1      | NK cell                      |
| KIR2DL1    | NK cell + NKT cell           |
| KIR2DL4    | NK cell + NKT cell           |
| KIR3DL2    | NK cell + NKT cell           |
| KIR3DL3    | NK cell + NKT cell           |
| KLHL30     | NK cell                      |
| KLRF1      | NK cell                      |
| NCR1       | NK cell + γδ T cell          |
| NCR2       | NK cell                      |

LUAD:

| Gene       | Description                  |
|------------|------------------------------|
| C17orf66   | NK cell + NKT cell           |
| CD244      | NK cell + NKT cell + γδ T cell|
| CDC20B     | NK cell + γδ T cell          |
| CMA1       | NK cell                      |
| CSF2       | NK cell + NKT cell + γδ T cell|
| FASLG      | NK cell + NKT cell           |
| KHDC1      | NK cell                      |
| KIR2DL1    | NK cell + NKT cell           |
| KIR2DL4    | NK cell + NKT cell           |
| KIR3DL2    | NK cell + NKT cell           |
| KIR3DL3    | NK cell + NKT cell           |
| KLHL30     | NK cell                      |
| KLRF1      | NK cell                      |
| NCR1       | NK cell + γδ T cell          |
| NCR2       | NK cell                      |
| Tumor Type | Gene  | Expression | Target Cell |
|------------|-------|------------|-------------|
| PRAD       | NCR3  | NK cell    |             |
|            | SH2D1B | NK cell+γδ T cell |             |
|            | CD244 | NK cell+NKT cell+γδ T cell |             |
|            | CLNK  | NK cell+γδ T cell |             |
|            | CMA1  | NK cell    |             |
|            | CSF2  | NK cell+NKT cell+γδ T cell |             |
|            | IKZF3 | NK cell+NKT cell |             |
|            | KIR2DL1 | NK cell+NKT cell |             |
|            | KIR2DL3 | NK cell+NKT cell |             |
|            | KIR2DL4 | NK cell+NKT cell |             |
|            | KIR3DL1 | NK cell+NKT cell |             |
|            | KIR3DL3 | NK cell+NKT cell |             |
|            | KLHL30 | NK cell    |             |
|            | KLRF1 | NK cell    |             |
|            | NCR2  | NK cell    |             |
|            | NCR3  | NK cell    |             |
|            | SH2D1B | NK cell+γδ T cell |             |
| STAD       | KLRF1 | NK cell    |             |
|            | NCR2  | NK cell    |             |
|            | NCR3  | NK cell    |             |
|            | C17orf66 | NK cell+NKT cell |             |
|            | KIR2DL1 | NK cell+NKT cell |             |
|            | KIR3DL1 | NK cell+NKT cell |             |
|            | KIR3DL3 | NK cell+NKT cell |             |
|            | CD244 | NK cell+NKT cell+γδ T cell |             |
|            | CDC20B | NK cell+γδ T cell |             |
|            | CLNK  | NK cell+γδ T cell |             |
|            | SH2D1B | NK cell+γδ T cell |             |
| UCEC       | CMA1  | NK cell    |             |

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|        | Cell Type                  |
|--------|---------------------------|
| KHDC1  | NK cell                   |
| KLRF1  | NK cell                   |
| NCR2   | NK cell                   |
| NCR3   | NK cell                   |
| C17orf66 | NK cell+NKT cell       |
| FASLG  | NK cell+NKT cell          |
| KIR2DL1 | NK cell+NKT cell          |
| KIR2DL3 | NK cell+NKT cell          |
| KIR2DL4 | NK cell+NKT cell          |
| KIR3DL1 | NK cell+NKT cell          |
| KIR3DL2 | NK cell+NKT cell          |
| CD244  | NK cell+NKT cell+γδ T cell|
| CSF2   | NK cell+NKT cell+γδ T cell|
| CLNK   | NK cell+γδ T cell         |
| SH2D1B | NK cell+γδ T cell         |
| ANGPT1 | NK cell+NKT cell          |

**SKCM**

|        | Cell Type                  |
|--------|---------------------------|
| C17orf66 | NK cell+NKT cell       |
| CLNK   | NK cell+γδ T cell         |
| CMA1   | NK cell                   |
| CSF2   | NK cell+NKT cell+γδ T cell|
| KIR3DL2 | NK cell+NKT cell          |
| KLHL30 | NK cell                   |
| KLRF1  | NK cell                   |
| NCR2   | NK cell                   |

**KIRC**

|        | Cell Type                  |
|--------|---------------------------|
| C17orf66 | NK cell+NKT cell       |
| CDC20B | NK cell+γδ T cell         |
| CLNK   | NK cell+γδ T cell         |
| CMA1   | NK cell                   |
| CSF2   | NK cell+NKT cell+γδ T cell|

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|          |          |          |
|----------|----------|----------|
| KHDC1    | NK cell  |          |
| KIR2DL1  | NK cell+NKT cell |          |
| KIR2DL3  | NK cell+NKT cell |          |
| KIR2DL4  | NK cell+NKT cell |          |
| KIR3DL2  | NK cell+NKT cell |          |
| KIR3DL3  | NK cell+NKT cell |          |
| KLHL30   | NK cell  |          |
| KLRF1    | NK cell  |          |
| NCR2     | NK cell  |          |
| STYK1    | NK cell+γδ T cell |          |
| THCA     |          |          |
| CHRNE    | NK cell  |          |
| CLNK     | NK cell+γδ T cell |          |
| CMA1     | NK cell  |          |
| CSF2     | NK cell+NKT cell+γδ T cell |          |
| FASLG    | NK cell+NKT cell |          |
| KIR2DL1  | NK cell+NKT cell |          |
| KIR2DL3  | NK cell+NKT cell |          |
| KIR3DL3  | NK cell+NKT cell |          |
| STYK1    | NK cell+γδ T cell |          |
**eTable 6. Experimental Evidence of the NK Cell Defective Genes for Tumor Surveillance**

| Gene | Tumor immune surveillance                                                                 | Reference |
|------|------------------------------------------------------------------------------------------|-----------|
| CD244| CD244 stimulates NK cell activation to overcome resistance of leukemia and neuroblastoma cells | 22        |
| NCR1 | Tumors in the absence of NCR1 grow faster in mice                                         | 23        |
### eTable 7. ITAM-Signaling Genes Associated With Patients’ Survival and Abundance of TILs in Cancers

| Cancer type | Gene       |
|-------------|------------|
| LGG         | ULBP2      |
|             | CD226      |
| BRCA        | ULBP3      |
|             | ITGB2      |
| HNSC        | PLCG2      |
|             | ITGB2      |
| BLCA        | ULBP2      |
| LUSC        | CD226      |
|             | HLA-E      |
| LUAD        | ICAM1      |
| PRAD        | CD226      |
|             | ICAM1      |
|             | ITGB2      |
|             | VAV1       |
| STAD        | PLCG1      |
|             | ULBP2      |
|             | ZAP70      |
| UCEC        | CD226      |
|             | PLCG2      |
|             | VAV1       |
| SKCM        | CD226      |
|             | ULBP1      |
|             | ULBP3      |
| KIRC        | HLA-E      |
| THCA        | PLCG1      |
|             | ULBP1      |
**eTable 8.** Abundance of TILs in Tumors Stratified by the Expression of NK Cell Ligand Genes of Tumors for the Bottom 10% and Top 10% of Patients Ranked by the Number of NK-Defective Genes

| Cancer | Cell type          | p-value (bottom 10%) | Ratio* | p-value (top 10%) | Ratio* |
|--------|--------------------|----------------------|--------|-------------------|--------|
| BLCA   | Activated CD8 T    | 5.9E-03              | 2.41   | NS                | -      |
|        | γδ T cell          | 4.9E-06              | 2.88   | NS                | -      |
|        | NK cell            | 6.6E-09              | 2.65   | 6.7E-03           | 1.26   |
|        | NKT cell           | 9.8E-12              | 1.27   | 1.0E-05           | 1.86   |
| BRCA   | Activated CD8 T    | 4.2E-02              | 1.16   | NS                | -      |
|        | γδ T cell          | 1.4E-02              | 1.43   | NS                | -      |
|        | NK cell            | 1.1E-04              | 1.35   | NS                | -      |
|        | NKT cell           | 2.1E-04              | 1.32   | NS                | -      |
| HNSC   | Activated CD8 T    | 8.7E-03              | 2.31   | NS                | -      |
|        | γδ T cell          | 1.9E-02              | 2.73   | NS                | -      |
|        | NK cell            | 1.4E-05              | 2.54   | NS                | -      |
|        | NKT cell           | 5.7E-04              | 2.01   | NS                | -      |
| KIRC   | Activated CD8 T    | NS                   | -      | NS                | -      |
|        | γδ T cell          | 3.8E-03              | 4.19   | NS                | -      |
|        | NK cell            | 9.6E-03              | 4.67   | NS                | -      |
|        | NKT cell           | 8.1E-03              | 19.98  | NS                | -      |
| LGG    | Activated CD8 T    | 4.2E-08              | 1.57   | NS                | -      |
|        | γδ T cell          | 1.4E-02              | 1.42   | 2.6E-02           | 1.36   |
|        | NK cell            | 3.5E-10              | 1.73   | NS                | -      |
|        | NKT cell           | 1.3E-15              | 2.81   | NS                | -      |
| LUAD   | Activated CD8 T    | NS                   | -      | NS                | -      |
|        | γδ T cell          | 3.2E-02              | 2.44   | 1.2E-02           | 1.05   |
|        | NK cell            | 1.1E-03              | 2.34   | 2.7E-03           | 1.09   |
| Tissue | Activated CD8 T | γδ T cell | NK cell | NKT cell | Activated CD8 T | γδ T cell | NK cell | NKT cell |
|--------|----------------|-----------|---------|----------|----------------|-----------|---------|----------|
| LUSC   | 2.4E-03        | 2.0E-04   | 1.2E-08 | 2.1E-09  | NS            | 2.4E-02   | 1.8E-03 | 2.2E-04  |
| PRAD   | NS            | 2.4E-02   | 1.8E-03 | NS       | NS            | 5.0E-01   | 8.0E-03 | 5.0E-03  |
| SKCM   | NS            | NS        | NS      | 3.2E-02  | NS            | NS        | NS      | NS       |
| STAD   | 1.8E-02        | 5.0E-01   | 8.0E-03 | 5.0E-03  | 2.5E-02        | 6.69      | 1.1E-02 | 1.3E-02  |
| THCA   | NS            | 2.5E-02   | 2.5E-02 | 3.2E-02  | NS            | NS        | NS      | NS       |
| UCEC   | NS            | NS        | NS      | NS       | NS            | NS        | NS      | NS       |

Note: ratio is the TIL’s abundance of the high-expression group/the low-expression group.
**eTable 9.** Clustering Analysis for the Melanoma (SKCM) and Gastric Cancer (STAD) Samples in Immune-Checkpoint Therapy (ICT) Trials

| Cancer type | Group                  | Number of responding patients | Number of non-responding | Enrichment ratio* |
|-------------|------------------------|-------------------------------|--------------------------|-------------------|
| SKCM        | TIME-rich              | 7                             | 22                       | 1.24              |
|             | TIME-intermediate/-poor| 3                             | 17                       | 0.69              |
| STAD        | TIME-rich              | 7                             | 11                       | 1.86              |
|             | TIME-intermediate/-poor| 5                             | 24                       | 0.61              |

*Ratio of the enrichment of responding patients in each group to that in all patient
eReferences

1. Bezman NA, Kim CC, Sun JC, et al. Molecular definition of the identity and activation of natural killer cells. *Nature immunology.* 2012;13(10):1000-1009.

2. Heng TS, Painter MW, Immunological Genome Project C. The Immunological Genome Project: networks of gene expression in immune cells. *Nature immunology.* 2008;9(10):1091-1094.

3. Koboldt DC, Zhang Q, Larson DE, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome research.* 2012;22(3):568-576.

4. Kircher M, Witten DM, Jain P, O’Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nature genetics.* 2014;46(3):310-315.

5. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics.* 2009;25(14):1754-1760.

6. Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics.* 2011;27(21):2987-2993.

7. Rock KL, Reits E, Neefjes J. Present Yourself! By MHC Class I and MHC Class II Molecules. *Trends in immunology.* 2016;37(11):724-737.

8. Charoentong P, Finotello F, Angelova M, et al. Pan-cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade. *Cell reports.* 2017;18(1):248-262.

9. Patel SJ, Sanjana NE, Kishon RJ, et al. Identification of essential genes for cancer immunotherapy. *Nature.* 2017;548(7669):537+.

10. Pan D, Kobayashi A, Jiang P, et al. A major chromatin regulator determines resistance of tumor cells to T cell-mediated killing. *Science.* 2018;359(6377):770+.

11. Brock G, Datta S, Pihur V, Datta S. clValid: An R package for cluster validation. *J Stat Softw.* 2008;25(4):1-22.

12. Zou J, Wang E. eTumorRisk, an algorithm predicts cancer risk based on co-mutated gene networks in an individual’s germline genome. *bioRxiv.* 2018:393090.

13. Okhuma T, Peters SAE, Woodward M. Sex differences in the association between diabetes and cancer: a systematic review and meta-analysis of 121 cohorts including 20 million individuals and one million events. *Diabetologia.* 2018;61(10):2140-2154.

14. Gross AI, Gallo JJ, Eaton WW. Depression and cancer risk: 24 years of follow-up of the Baltimore Epidemiologic Catchment Area sample. *Cancer causes & control : CCC.* 2010;21(2):191-199.

15. Batty GD, Russ TC, Stamatakis E, Kivimaki M. Psychological distress in relation to site specific cancer mortality: pooling of unpublished data from 16 prospective cohort studies. *Bmj.* 2017;356:j108.

16. Bahr I, Jahn J, Zipprich A, Pahlow I, Spielmann J, Kielstein H. Impaired natural killer cell subset phenotypes in human obesity. *Immunologic research.* 2018;66(2):234-244.

17. O’Shea D, Cawood TJ, O’Farrelly C, Lynch L. Natural killer cells in obesity: impaired function and increased susceptibility to the effects of cigarette smoke. *PloS one.* 2010;5(1):e8660.

18. Wyman PA, Moynihan J, Eberly S, et al. Association of family stress with natural killer cell activity and the frequency of illnesses in children. *Archives of pediatrics & adolescent medicine.* 2007;161(3):228-234.
19. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. *Nature*. 2015;523(7559):231-235.

20. Grasso CS, Giannakis M, Wells DK, et al. Genetic Mechanisms of Immune Evasion in Colorectal Cancer. *Cancer discovery*. 2018;8(6):730-749.

21. Bjorkstrom NK, Ljunggren HG, Michaelsson J. Emerging insights into natural killer cells in human peripheral tissues. *Nature reviews Immunology*. 2016;16(5):310-320.

22. Altvater B, Landmeier S, Pscherer S, et al. 2B4 (CD244) signaling by recombinant antigen-specific chimeric receptors costimulates natural killer cell activation to leukemia and neuroblastoma cells. *Clin Cancer Res*. 2009;15(15):4857-4866.

23. Elboim M, Gazit R, Gur C, Ghadially H, Betser-Cohen G, Mandelboim O. Tumor immunoediting by NKp46. *J Immunol*. 2010;184(10):5637-5644.