

CD 80 and CD 86 Expression, Clinical Implications Are Cancer Dependent as Revealed Through Pan-cancer Analysis

Ayobami Matthew Olajuyin (✉ ayobami.olajuyin@aun.edu.ng)

Henan Provincial People Hospital <https://orcid.org/0000-0002-1733-6016>

Sharon Ibialate Georgewill

American University of Nigeria School of Information Technology and Computing

Adefunke Kafayat Olajuyin

AUN

Jamiyu Ayodeji Saliu

AAUA

Malachy I. Okeke

AUN

Hayatu M. Raji

AUN

Jennifer Tyndall

AUN

Xia Guo

UT

Primary research

Keywords: CD 86, CD 80, Lymphoma, Leukemia, TCGA, Biomarker

Posted Date: June 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-606743/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Cluster of Differentiation 80 and CD 86 can also be called B7-1 and B7-2 respectively. They are proteins fundamentally expressed on antigen-presenting cells (APCs), including induced dendritic cells (IDCs), langerhans cells, germinal center dendritic cells (GCDCs), activated monocytes, macrophages and B-cells. They are considered to be a possible therapeutic target and biomarker of great significance. However, there are still inconsistent pieces of information and their clinical importance is yet to be established.

Methods

Here we investigated CD 80 and 86 as biomarkers by utilizing several large genomic data collections. (The Cancer Genome Atlas, Cancer Cell Line Encyclopedia, Quantitative proteomics Cancer cell line Encyclopedia Genotype-Tissue Expression,) and analyzed CD 80 and CD 86 expression in thousands of normal and cancer samples and cell lines along with their clinical survival analysis.

Results

This study presented that CD 86 was expressed more in post-treatment blood cancer in the blood and post-treatment blood cancer in the bone marrow while it was expressed least in normal tissues and cell lines. The Hodgkin lymphoma cell line L428 cell lysate illustrated that there was a high relative protein expression of 6.6 for the CD 86 gene. it indicated that cancer in the esophagus had the highest copy number value and indicated a medium level amplification of the CD 86 gene and prostate cancer had a hemizygous deletion of the CD 86 gene with the least copy number value. Furthermore, on the non-Hodgkin lymphoma cell line REC1, illustrated the highest relative protein expression of the CD 86 gene among the other types of cancer cell line, its protein expression value was 8.19. Also, for cancer type leukemia, the subtype acute myeloid leukemia showed a significant relative protein expression. The acute myeloid leukemia cell line EOL1 indicated that there was a high relative protein expression of 6.5. However, the protein expression for CD 80 is yet to be elucidated.

Conclusions

Taken together, CD 80 and 86 may be potential biomarkers of great clinical significance. The Kaplan Meier plots unveiled that CD 86 and CD 80 were significantly associated with overall survival analysis in the Large B-cell lymphoma, and the different tumor types.

Background

CD 86 (B7.2) gene is essential for transplantation, autoimmunity, and tumor immunity (1, 2). CD 86 specifically functions as a ligand that links two proteins together including the CD 28 antigen and the cytotoxic T-lymphocyte associated protein 4, the joining of CD 86 to CD 28 antigen is a costimulatory signal for the activation of the T-cell (3). Hence, when CD 86 protein is coupled to the cytotoxic T-

lymphocyte associated protein 4 (CTLA-4), when this happens the T-cell activation is regulated negatively and the body receives a decreased immune response (4) CTLA-4 is a tremendous endocytic molecule, its residence time on the cell surface is very limited, genes for CD 28 and CTLA-4 are both expressed similarly on the chromosome and they share homology of about 30% at the amino acid level indicating that CD 28 and CTLA-4 have evolved from the same ancestral gene. Whereas CD 28 is constitutively expressed on the surface of most T cells, CTLA-4 expression is limited to activated conventional T cells and more importantly, T-regs thus, high expression of CD 86 proteins (5).

CD 80, which is alternatively called also B7.1, is a coregulatory receptor expressed on the activated T cells, dendritic cells, macrophages, monocytes which are seen on the surface of antigen presenting cells (6, 7). It can also be found on the myeloid-derived suppressor cells (8). Previous investigations unveiled the importance of CD28/B7 family of coregulatory receptors, and that CD 80 is associated with regulation of the immune system (9). Hence, it is capable of enhancing tumor progression in the tumor microenvironment by impeding the immune response of the tumor (4). The expression of CD 86 is low and upregulated quickly on the APC while that of CD 80 is inducibly expressed later than CD 86. The costimulatory molecules on APCs have been investigated extensively meanwhile their functions on the T cells are yet to be elucidated (4).

CD 80 and CD 86 investigations in cancer biology have been vigorous and many researchers unveil its possible therapeutic target. Elevated CD 80 and CD 86 expression have been documented in many cancer types apart from blood cancer (lymphoma and leukemia) which is connected to tumor progression, poor prognosis and metastasis in different cancers such as, breast cancer (10), pancreatic cancer (11), hepatocellular carcinoma (12), colorectal cancer (13, 14), gastric cancer (15), esophageal carcinoma (16), renal cell cancer (17, 18), ovarian cancer (19), gallbladder adenocarcinoma (20), kaposi sarcoma (21), Ewing sarcoma (22–24), lung cancer (25), head and neck carcinoma, nasopharyngeal carcinoma (26, 27), thyroid cancer (28, 29).

A biomarker is a biological molecule such as a protein, DNA, RNA, or circulating extracellular vesicles (EVs) that can be found in blood, biological fluids and tissues and is an indicator of a normal physiologic or a diseased state, biomarkers are used for molecular diagnosis, patient prognosis and to determine the outcome of the targeted therapy (30). Over the years, cancer researchers have invested their time and resources in the early detection of cancer, however, the diagnosis of cancer has relied on the microanatomy of tissues (31). Cancer researchers have been able to use biomarkers for early detection of tumors, and vigorous research is ongoing to improve the use of biomarkers such as being able to use biomarkers to predict tumor outcome and to predict the response of patients with tumors to particular therapeutic drugs (32). There are various types of biomarkers, which includes diagnostic, prognostic, pharmacodynamic, predictive, safety, and monitor etc (30, 32).

Taken together, we utilized the database of The Cancer Genome Atlas (TCGA; <https://portal.gdc.cancer.gov/>), Cancer Cell Line Encyclopedia (CCLE; <https://portals.broadinstitute.org/ccle/>), DepMap data explorer (<http://depmap.org/portal/interactive>), we thoroughly

evaluated the expression pattern of CD 80 and CD 86. Hence, we comprehensively analyzed CD 86 and CD 80 as biomarkers of clinical significance. We utilized the protein expression, copy number, gene expression, mRNA and survival analysis of different tumor types. To our knowledge, this is the first investigation to unveil the comprehensive analysis of CD 80 and CD 86 expression and implications are cancer dependent.

Materials And Methods

The Cancer Genome Atlas, Cancer Cell Line Encyclopedia, Genotype-tissue expression, Quantitative Proteomic Cancer Cell Line Encyclopedia R-software incorporated into the Xena browser, Human protein atlas, Human proteomics Database were used in the investigation. The investigation was done using a substantial genomic data collection such as The Cancer Genome Atlas, Cancer Cell Line Encyclopedia Genotype-tissue expression and Quantitative proteomic cancer cell line encyclopedia to extensively look over CD86 and CD80 expression in a wide range of normal and lung, lymphoma and leukemia and different cancer samples, cell lines, along with their clinical phenotypes. We utilized the statistical methods of Kaplan Meier method and compared how expression of CD86 and CD 80 were correlated with the survival rate in, lung and lymphoma cancer types and other tumor types.

Data extraction from TCGA Database: All cases of patients with, large B-cell lymphoma,, Lung adenocarcinoma cell and other tumor types were accessed from the TCGA database, a database maintained by the National Cancer Institute and the National Human Genome Research Institute, covering approximately 28 % of the U.S. population.

DATA PROCESSING AND ANALYSIS

Clinical survival data of lung, lymphoma and leukemia patients from TCGA were downloaded from the database. We utilized the Xena browser in which R software is incorporated. We analyzed our overall survival analysis using Kaplan Meiers plot.

Discussion

The importance of CD 80 and CD 86 in cancer biology and clinical implications have fascinated lots of consideration. Many investigations have been done by the previous researchers however, some of the investigations have the small sample size and there is still inconsistent information and its clinical importance is yet to be established. The expression of CD 86 in different cancer sample types showed that CD 86 was expressed more in post-treatment blood cancer in the blood and post-treatment blood cancer in the bone marrow while it was significantly different from expression in cell line and control analyte. Hence, CD 86 may be a possible biomarker in the detection of blood cancer. To comprehend the expression and distribution of CD 86 in normal human tissues, we examined its expression across 19,131 samples from the TCGA TARGET GTEX data. As shown in Fig. 1a, we discovered that the expression of CD 86 in different cancer sample types was significantly different from the control analyte $P < 0.05$.

CD 80 gene expression between different cancers and tumor, cell line, control analyte, and their normal tissues were highly dependent on cancer and tumor types. We investigated CD 80 expression on different types of cancer from TCGA Target GTEX. All tumors had a certain level of expression but the expression in primary blood-derived cancer (peripheral blood and bone marrow), was higher than other cancer types (Fig. 1b). Blood cancer (leukemia) have been discovered to have different diagnosis, prognosis and molecular mechanisms (33, 34). There are different types of blood cancer which includes myeloid neoplasms, lymphoid neoplasms (leukemias and lymphomas), and myeloproliferative disorders (35) The CD 80 expression in this tumor in the TCGA Target GTEX dataset indicates mRNA expression is an important quantity for CD 80 expression and shows its significant role in blood cancer. The P- value ($P < 0.05$) was significantly different from the control analyte. Hence CD 80 may be a potential biomarker for the diagnosis, prognosis and prediction of blood cancer.

Most previous investigations utilized Immunohistochemistry (IHC) to detect CD 86 and CD 80 (36). Protein expression may be determined using the IHC while mRNA expression from RNA may be crucial in the discovery of novel biomarkers (37) and therapeutic targets (38). The correlation between mRNA and protein expression is not fully elucidated. Here, this investigation, utilized the mRNA to unveils the mRNA expression of the CD 80 and CD 86 using the TCGA database. The Burkitt lymphoma was one of the tumors with very high expression of CD 86 mRNA and B-cell lymphoma in CCLE samples; however, its expression was low in lungs (NSCLC), liver, thyroid, ovary, glioma as shown in Fig. 2a. The expression of CD 80 mRNA was high in lymphoma, and Burkitt lymphoma cancer cell lines and was low in urinary tract, thyroid, osteosarcoma, and meningioma cancer cell lines respectively as shown in Fig. 2b. Hence, CD 86 and CD 80 may be useful as biomarker and the expression are in consonant with previous investigations (39)

As shown in Fig. 3a, it showed that cancer in the esophagus had the highest copy number value and showed a medium level amplification of CD 86 gene and prostate cancer seems to almost have a hemizygous deletion of the CD 86 gene with the least copy number value. The copy number of CD 86 was in a medium amplification in lung small cell carcinoma, lung non-small cell carcinoma, B- cell lymphoma and T-cell lymphoma. This indicated that CD 86 was moderately expressed in lung cancer and lymphoma and therefore may be used for detection in lung cancer and lymphoma.

The CD 86 relative protein expression on different cancer cell lines (including the types and subtypes) was investigated. CD 86 relative protein expression on these cancer cell lines as shown in the Fig. 4. Hodgkin lymphoma showed a significant relative protein expression. The Hodgkin lymphoma cell line L428 cell lysate illustrated that there was a high relative protein expression of 6.6 for the CD 86 gene meaning that CD 86 may be possible biomarker. Furthermore, on the non-Hodgkin lymphoma cell line REC1 illustrated the highest relative protein expression of the CD 86 gene among the other types of cancer cell line, its protein expression value was 8.19. Also, for the cancer type leukemia, the subtype acute myeloid leukemia displayed a significant relative protein expression. The acute myeloid leukemia cell line EOL1 demonstrated that there was a high relative protein expression of 6.5. however, CD 80 the relative protein expression of different cancer cell lines is yet to be investigated.

Furthermore, we used the Kaplan Meier survival analysis to unveil the importance of CD 86 and CD 80 as potential biomarker. From the Kaplan Meier plots, using the different types of tumor from the TCGA and Target Pan Cancer. Hence, the biomarkers were significantly associated with overall survival with the different types of tumors. We also used the Large B-cell lymphoma and discovered that the CD 86 and CD 80 were significantly associated with overall survival of the patients (Fig. 5a-d). Hence, they may be possible biomarker and therapeutic target (38, 40).

Conclusion And Future Perspective

In conclusion, our analysis unveiled that CD 86 and CD 80 are actually highly expressed in some tumors. The Kaplan Meier survival analysis unveiled that CD86 and CD 80 were significantly associated with overall survival analysis in the Large B-cell lymphoma. Hence, they may be potential biomarker of clinical importance in cancer. However, there is need for further investigations in the proteomics of CD 80 which is yet to be elucidated.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets used and analyzed during the current study are available from The Cancer Genome Atlas (portal.gdc.cancer.gov), Cancer Cell Line Encyclopedia (<https://portals.broadinstitute.org/ccle/>), Quantitative proteomics Cancer cell line Encyclopedia (<http://depmap.org/portal/interactive>) Genotype-Tissue Expression, All the results will be available at reasonable request

Competing interests

The authors declare that they have no competing interests

Funding

Not applicable

Authors contribution

All the authors contributed in the preparation of this paper. AMO and SIG were responsible for data collection, analysis and drafting of the article. AKO, JAS, OIM, HMR and TJ were responsible for data

analysis. AMO and GX made substantial contributions to manuscript conception and design and participated in its critical review and final editing. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

References

1. Guinan EC, Gribben JG, Boussiotis VA, Freeman GJ, Nadler LM. Pivotal role of the B7: CD28 pathway in transplantation tolerance and tumor immunity. 1994.
2. Yamada A, Salama AD, Sayegh MH. The role of novel T cell costimulatory pathways in autoimmunity and transplantation. *J Am Soc Nephrol*. 2002;13(2):559–75.
3. Collins M, Ling V, Carreno BM. The B7 family of immune-regulatory ligands. *Genome biology*. 2005;6(6):1–7.
4. Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. *Immunity*. 2007;27(1):111–22.
5. Azimzadeh P, Romani S, Mirtalebi H, Fatemi SR, Kazemian S, Khanyaghma M, et al. Association of co-stimulatory human B-lymphocyte antigen B7-2 (CD86) gene polymorphism with colorectal cancer risk. *Gastroenterology Hepatology from bed to bench*. 2013;6(2):86.
6. Azuma M, Yssel H, Phillips JH, Spits H, Lanier LL. Functional expression of B7/BB1 on activated T lymphocytes. *The Journal of experimental medicine*. 1993;177(3):845–50.
7. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol*. 2005;23:515–48.
8. Poschke I, Mougiakakos D, Hansson J, Masucci GV, Kiessling R. Immature immunosuppressive CD14 + HLA-DR⁻/low cells in melanoma patients are Stat3hi and overexpress CD80, CD83, and DC-sign. *Cancer research*. 2010;70(11):4335–45.
9. Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, et al. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nature medicine*. 2003;9(5):562–7.
10. Olesch C, Sha W, Angioni C, Sha LK, Acaf E, Patrignani P, et al. MPGES-1-derived PGE2 suppresses CD80 expression on tumor-associated phagocytes to inhibit anti-tumor immune responses in breast cancer. *Oncotarget*. 2015;6(12):10284.
11. Bengsch F, Knoblock DM, Liu A, McAllister F, Beatty GL. CTLA-4/CD80 pathway regulates T cell infiltration into pancreatic cancer. *Cancer Immunol Immunother*. 2017;66(12):1609–17.
12. Zhai S, Liu J, Zhu P. Expression of CD80 and CD86 in hepatocellular carcinoma and liver cirrhosis tissues. *J Fourth Mil Med Univ*. 2000;21(3):26–7.
13. DOEBERITZ K. Gene transfer of costimulatory molecules into a human colorectal cancer cell line: requirement of CD54, CD80 and class II MHC expression for enhanced immunogenicity. *Immunology*.

- 1998;93(3):390–7.
14. Wu D, Tang R, Qi Q, Zhou X, Zhou H, Mao Y, et al. Five functional polymorphisms of B7/CD28 co-signaling molecules alter susceptibility to colorectal cancer. *Cellular immunology*. 2015;293(1):41–8.
 15. Koyama S, Maruyama T, Adachi S, Nozue M. Expression of costimulatory molecules, B7-1 and B7-2 on human gastric carcinoma. *J Cancer Res Clin Oncol*. 1998;124(7):383–8.
 16. Yang W-F, Yu J-M, Zuo W-S, Wang S-Z. Expression of CD80, CD86, TGF-beta1 and IL-10 mRNA in the esophageal carcinoma. *Zhonghua zhong liu za zhi [Chinese journal of oncology]*. 2006;28(10):762–5.
 17. Flörcken A, Johannsen M, Nguyen-Hoai T, Gerhardt A, Miller K, Dörken B, et al. Immunomodulatory molecules in renal cell cancer: CD80 and CD86 are expressed on tumor cells. *International Journal of Clinical Experimental Pathology*. 2017;10(2):1443–54.
 18. Westermann J, Flörcken A, Willimsky G, Van Lessen A, Kopp J, Takvorian A, et al. Allogeneic gene-modified tumor cells (RCC-26/IL-7/CD80) as a vaccine in patients with metastatic renal cell cancer: a clinical phase-I study. *Gene therapy*. 2011;18(4):354–63.
 19. Melichar B, Nash M, Lenzi R, Platsoucas C, Freedman R. Expression of costimulatory molecules CD80 and CD86 and their receptors CD28, CTLA-4 on malignant ascites CD3 + tumour-infiltrating lymphocytes (TIL) from patients with ovarian and other types of peritoneal carcinomatosis. *Clinical Experimental Immunology*. 2000;119(1):19–27.
 20. Ghidini M, Cascione L, Carotenuto P, Lampis A, Trevisani F, Previdi MC, et al. Characterisation of the immune-related transcriptome in resected biliary tract cancers. *Eur J Cancer*. 2017;86:158–65.
 21. Foreman KE, Wrone-Smith T, Krueger AE, Nickoloff BJ. Expression of costimulatory molecules CD80 and/or CD86 by a Kaposi's sarcoma tumor cell line induces differential T-cell activation and proliferation. *Clinical Immunology*. 1999;91(3):345–53.
 22. Mahlendorf DE, Staeger MS. Characterization of Ewing sarcoma associated cancer/testis antigens. *Cancer Biol Ther*. 2013;14(3):254–61.
 23. Tang S, Guo W, Guo Y, Qu H, Li D. In vitro antitumor immune response induced by fusion of dendritic cells and Ewing's sarcoma cells. *Zhonghua wai ke za zhi [Chinese Journal of Surgery]*. 2005;43(12):803–6.
 24. Wang J, Zhou Y, Feng D, Yang H, Li F, Cao Q, et al. CD86 + 1057G/A Polymorphism and susceptibility to Ewing's sarcoma: A Case–Control Study. *DNA cell biology*. 2012;31(4):537–40.
 25. Chen C, Qu Q-X, Shen Y, Mu C-Y, Zhu Y-B, Zhang X-G, et al. Induced expression of B7-H4 on the surface of lung cancer cell by the tumor-associated macrophages: a potential mechanism of immune escape. *Cancer letters*. 2012;317(1):99–105.
 26. Chang C-S, Chang JH, Hsu NC, Lin H-Y, Chung C-Y. Expression of CD80 and CD86 costimulatory molecules are potential markers for better survival in nasopharyngeal carcinoma. *BMC Cancer*. 2007;7(1):1–7.
 27. Su H, Luo Q, Xie H, Huang X, Ni Y, Mou Y, et al. Therapeutic antitumor efficacy of tumor-derived autophagosome (DRibble) vaccine on head and neck cancer. *Int J Nanomed*. 2015;10:1921.

28. Xu W-C, Li Z-B, Chen Y-R, Li X-T, Huang J-X, Li Y-G, et al. Expression and distribution of S-100, CD83, and costimulatory molecules (CD80 and CD86) in tissues of thyroid papillary carcinoma. *Cancer investigation*. 2011;29(4):286–92.
29. Tuccilli C, Baldini E, Sorrenti S, Catania A, Antonelli A, Fallahi P, et al. CTLA-4 and PD-1 ligand gene expression in epithelial thyroid cancers. *International journal of endocrinology*. 2018;2018.
30. Califf RM. Biomarker definitions and their applications. *Experimental Biology Medicine*. 2018;243(3):213–21.
31. Spizzo G, Fong D, Wurm M, Ensinger C, Obrist P, Hofer C, et al. EpCAM expression in primary tumour tissues and metastases: an immunohistochemical analysis. *J Clin Pathol*. 2011;64(5):415–20.
32. Hayes DF. Biomarker validation and testing. *Molecular oncology*. 2015;9(5):960–6.
33. Pui C-H, Relling MV, Downing JR. Acute lymphoblastic leukemia. *N Engl J Med*. 2004;350(15):1535–48.
34. Sawyers CL. Chronic myeloid leukemia. *N Engl J Med*. 1999;340(17):1330–40.
35. Dash D, Janasik M. Types of blood cancers-and new molecular diagnostics. *Med Lab Obs*. 2013;45(8):8–12.
36. Said EA, Al-Reesi I, Al-Riyami M, Al-Naamani K, Al-Sinawi S, Al-Balushi MS, et al. Increased CD86 but not CD80 and PD-L1 expression on liver CD68 + cells during chronic HBV infection. *PLoS One*. 2016;11(6):e0158265.
37. Koussounadis A, Langdon SP, Um IH, Harrison DJ, Smith VA. Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. *Scientific reports*. 2015;5(1):1–9.
38. Haabeth OAW, Blake TR, McKinlay CJ, Tveita AA, Sallets A, Waymouth RM, et al. Local delivery of Ox40l, Cd80, and Cd86 mRNA kindles global anticancer immunity. *Cancer research*. 2019;79(7):1624–34.
39. Zheng Z, Takahashi M, Aoki S, Toba K, Liu A, Osman Y, et al. Expression patterns of costimulatory molecules on cells derived from human hematological malignancies. *Journal of experimental clinical cancer research: CR*. 1998;17(3):251–8.
40. Stopeck AT, Gessner A, Miller TP, Hersh EM, Johnson CS, Cui H, et al. Loss of B7. 2 (CD86) and intracellular adhesion molecule 1 (CD54) expression is associated with decreased tumor-infiltrating T lymphocytes in diffuse B-cell large-cell lymphoma. *Clinical cancer research*. 2000;6(10):3904–9.

Figures

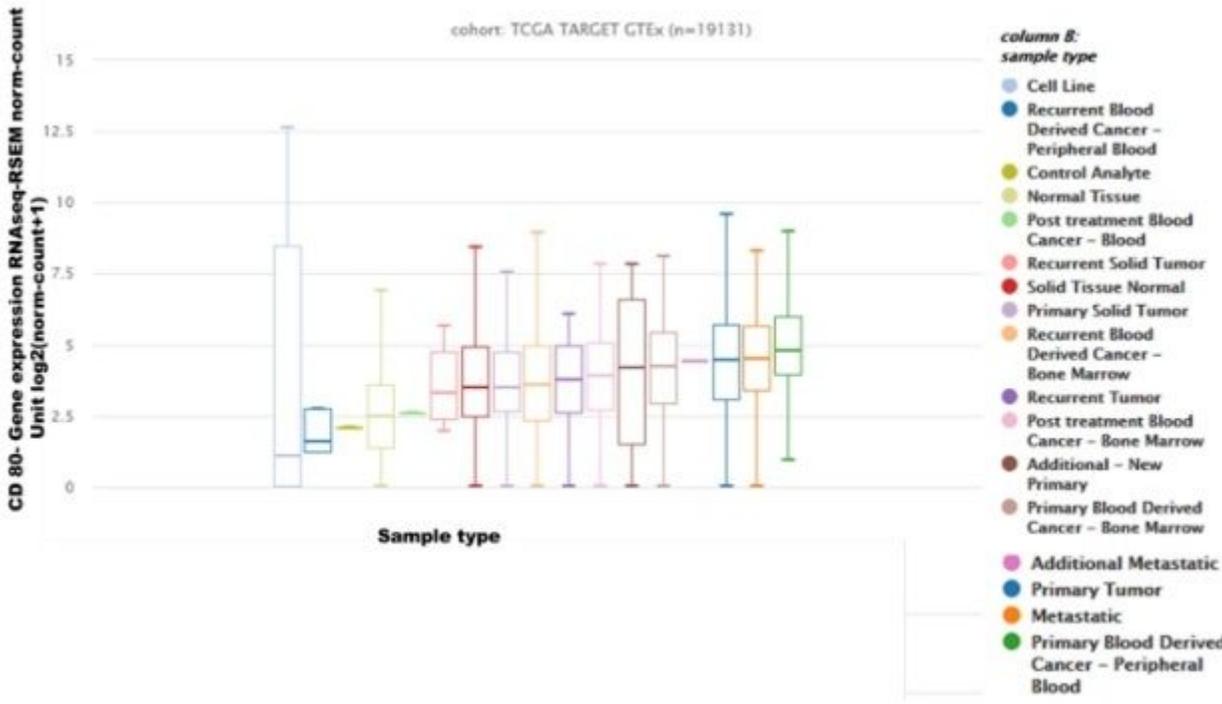
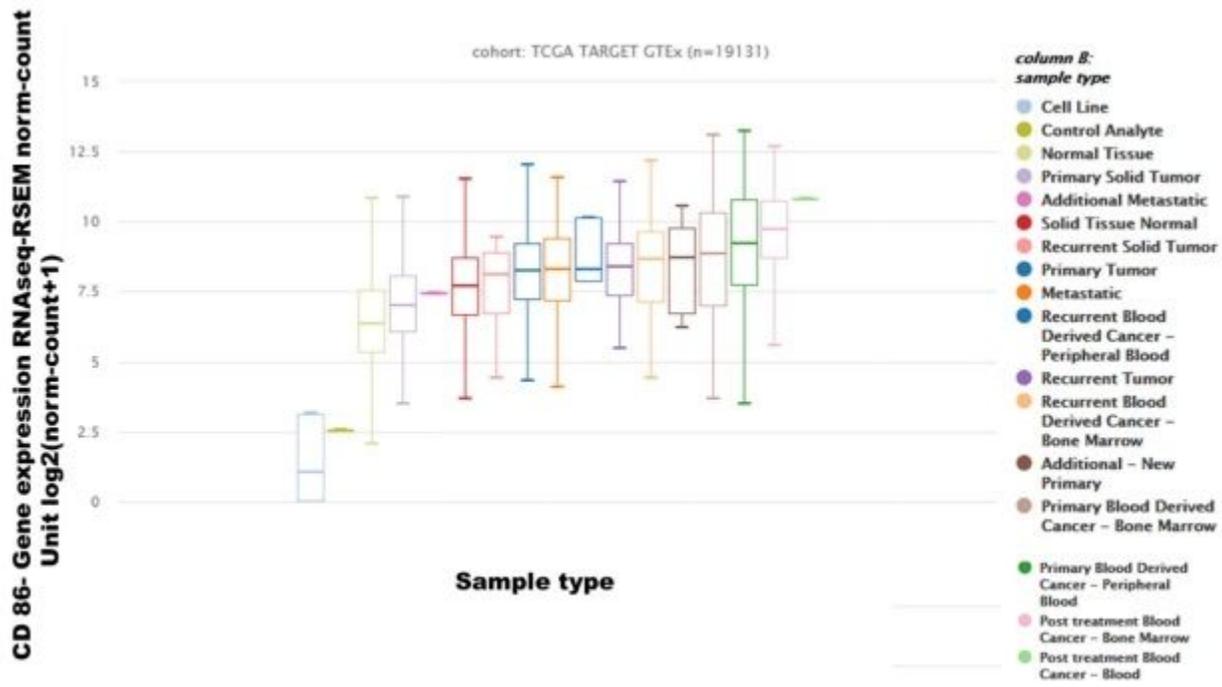


Figure 1

a: CD 86 gene expression between tumor and normal samples in TCGA Target GTEx data type (A) Boxplot for CD 80 expression across different cancers. Y-axis is the log2 expression range where the bar represents median expression of tumors or normal and lower and upper box ends represent the 25 and 75 percentile expression b: CD 80 gene expression between tumor and normal samples in TCGA Target GTEx data. (A) Boxplot for CD 80 expression across different cancers. Y-axis is the log2 expression range where

the bar represents median expression of tumors or normal and lower and upper box ends represent the 25 and 75 percentile expression

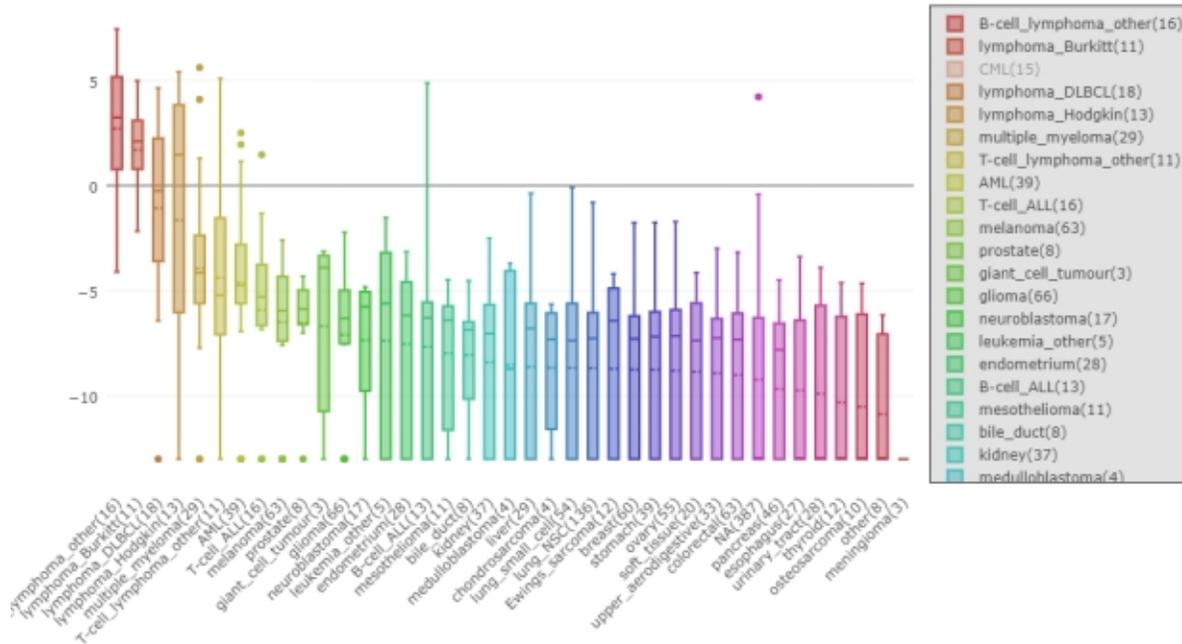
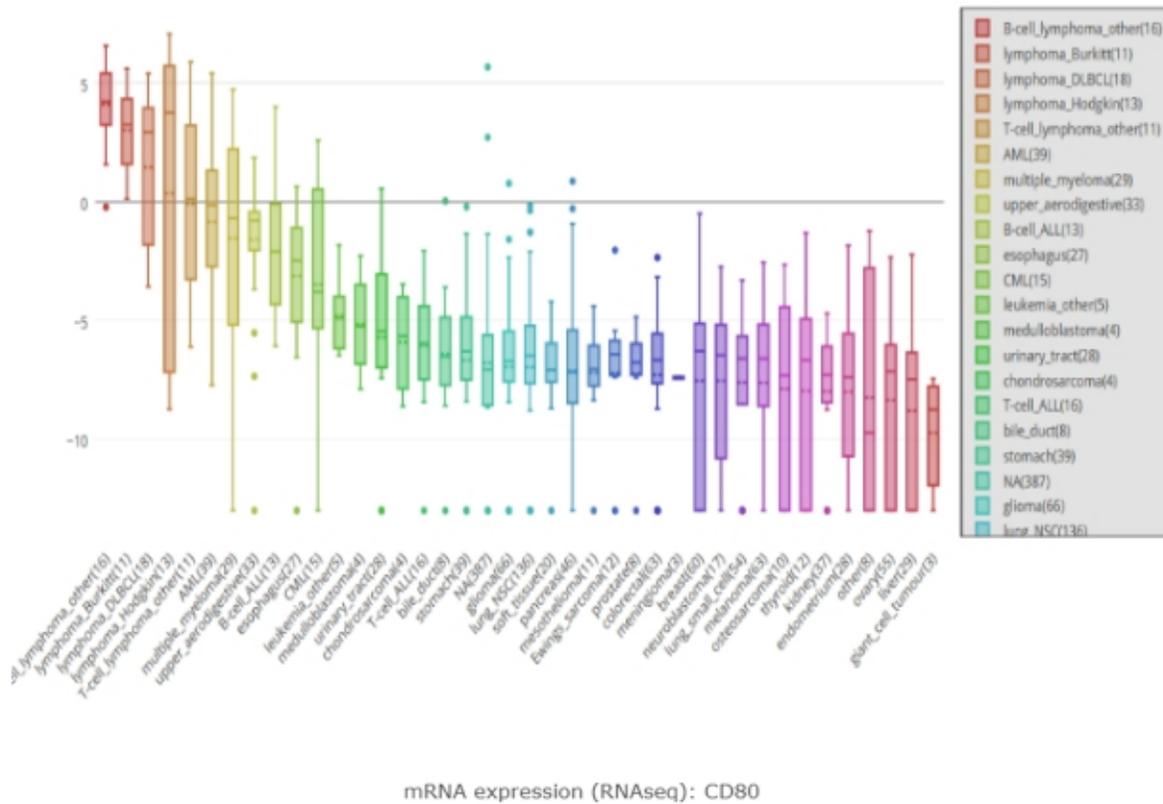
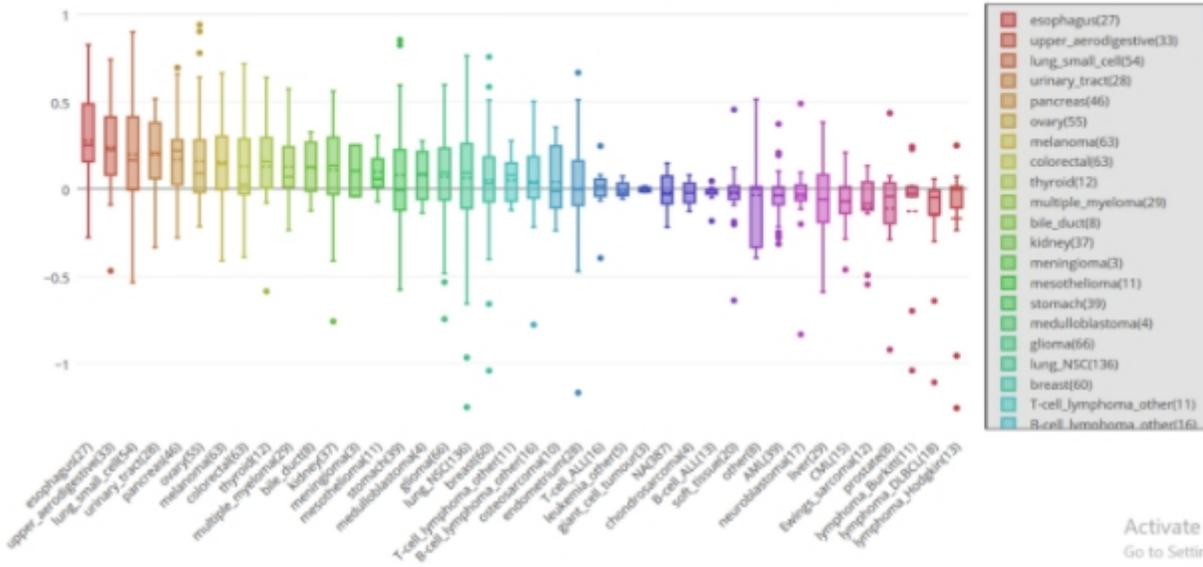


Figure 2

a: The mRNA expression of CD 86 on different types of cancer cell lines b: The expression of CD 80 mRNA on different cancer cell lines.

Copy Number: CD86



Activate Win
Go to Settings to

Copy Number: CD80

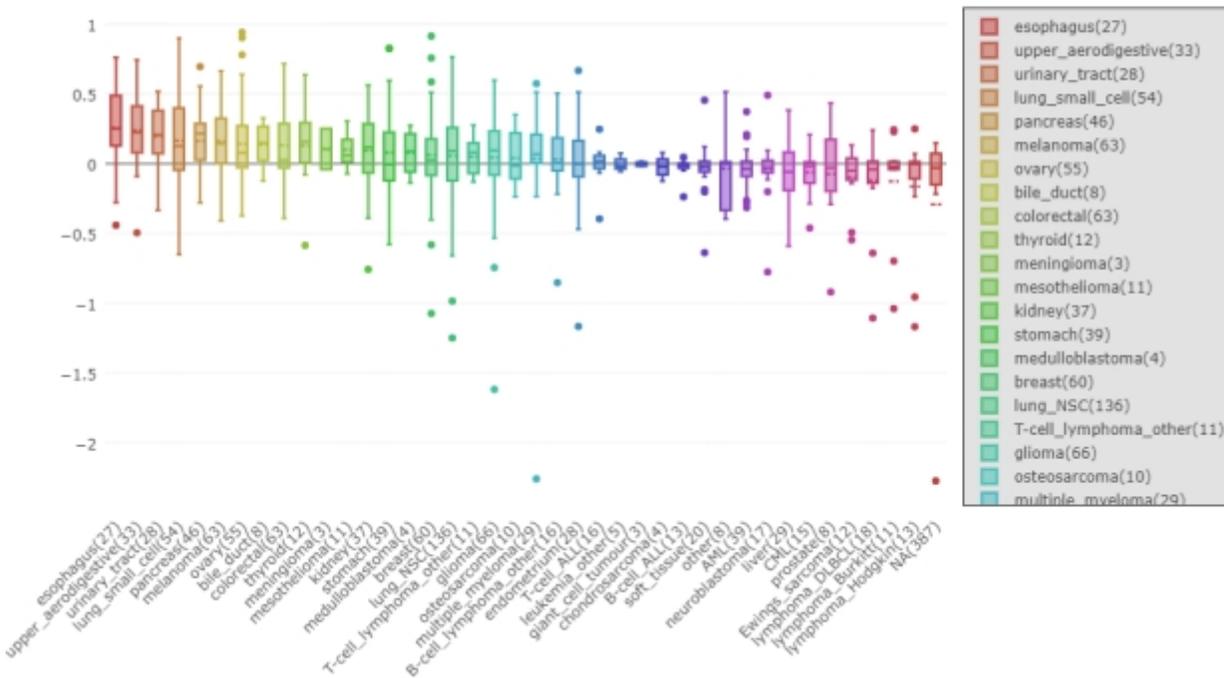


Figure 3

a: Copy number CD 86 on different cancer cell lines. Copy number values include -1= homozygous deletion, -0.5= hemizygous deletion, 0= neutral, 0.5= medium level amplification, 1= high level amplification. b: Copy number CD 86 on different cancer cell lines. Copy number values include -1= homozygous deletion, -0.5= hemizygous deletion, 0= neutral, 0.5= medium level amplification, 1= high level amplification.

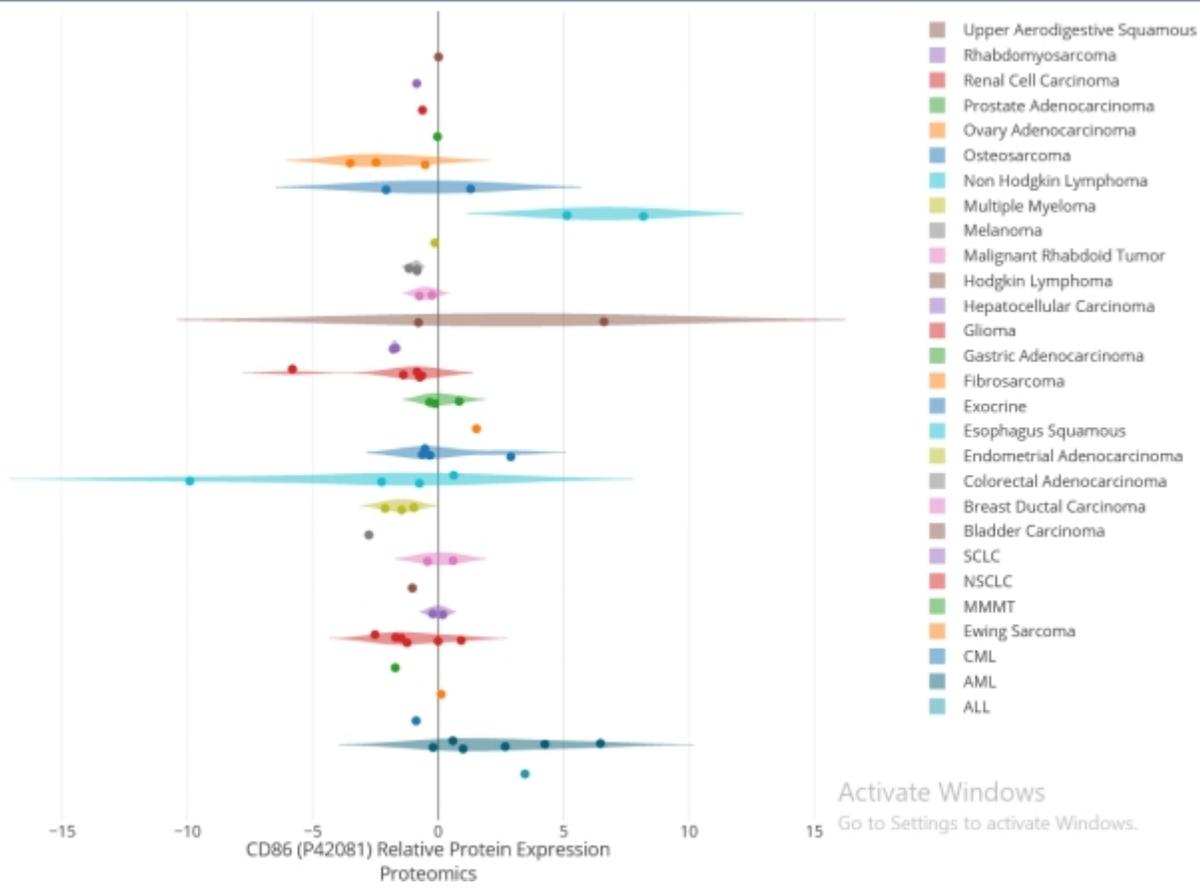


Figure 4

The CD 86 relative protein expression on different cancer cell lines (including the types and subtypes).

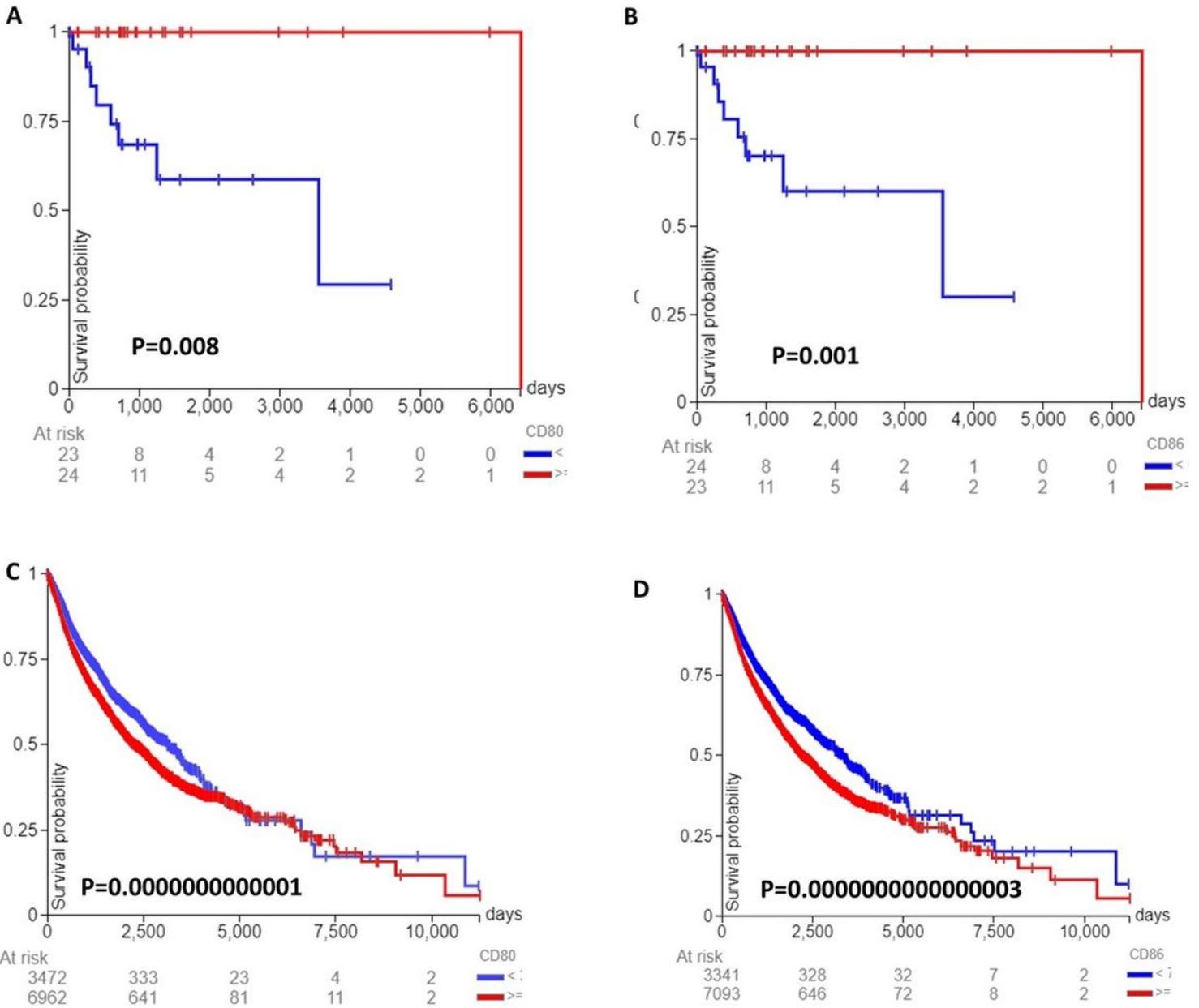


Figure 5

Kaplan Meier curve associated with Overall survival: A and B, CD 80 and CD86 KM plot on Large B-cell lymphoma ($p < 0.008$), $p < 0.0001$ respectively. C and D, CD 80 and CD 86 KM plot on TCGA Target GTEX showing different tumors $P < 0.000000000001$, $P < 0.000000000000003$ respectively