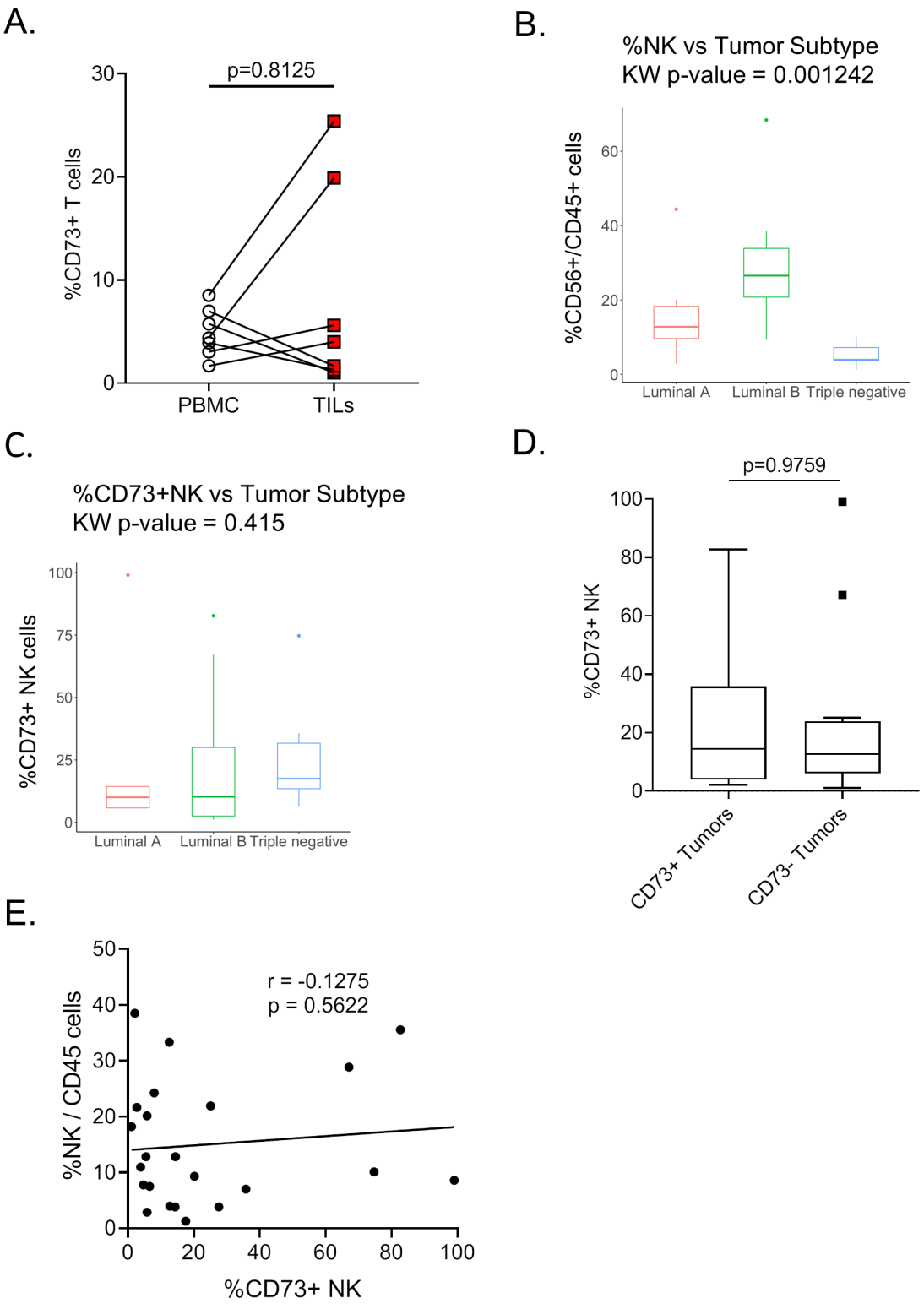
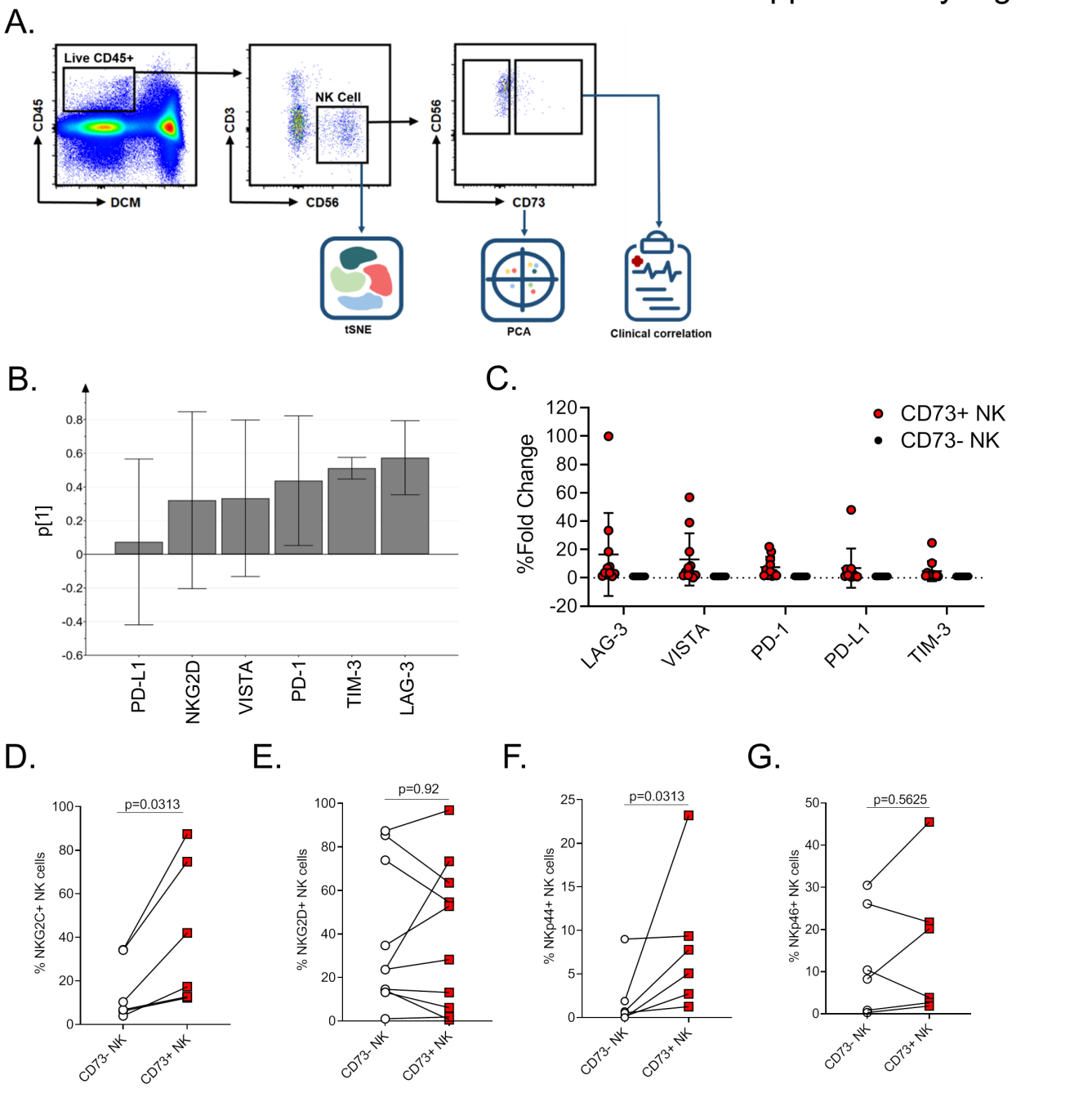
Supplementary Figure 1



Supplementary figure 1.

Correlation of immune-phenotyping with clinical parameters. **A,** Differential expression of CD73 by NK cells from peripheral blood versus tumor resections for sarcoma. (n=7) Wilcoxon signed rank test was done to test for significance in matching data points. **B,** Percentage of CD56+, CD3- cells out of CD45+ cells over three defined molecular subtypes of breast cancer tumor cohort collected shown in table 1. **C,** Percentage of CD73+ NK cells out of total NK cells (CD56+, CD45+ and CD3-) over 3 defined molecular subtypes of breast cancer tumor cohort collected shown in table 1. Kruskal-Wallis test was used to test for significant correlations in both figure A and B. **D,** CD73+ NK cells out of total NK cells over CD73 expression in CD45- tumor cells (>1% as cut-off) Mann-Whitney U rank test was used to test for significance. **E,** Spearman correlation coefficient to test relationship between percentage of NK cells out of CD45+ cells over percentage of CD73+ NK cells out of total NK cells. Clinical correlation in figures B to E were done in breast cancer cohort with sample size, n=25.

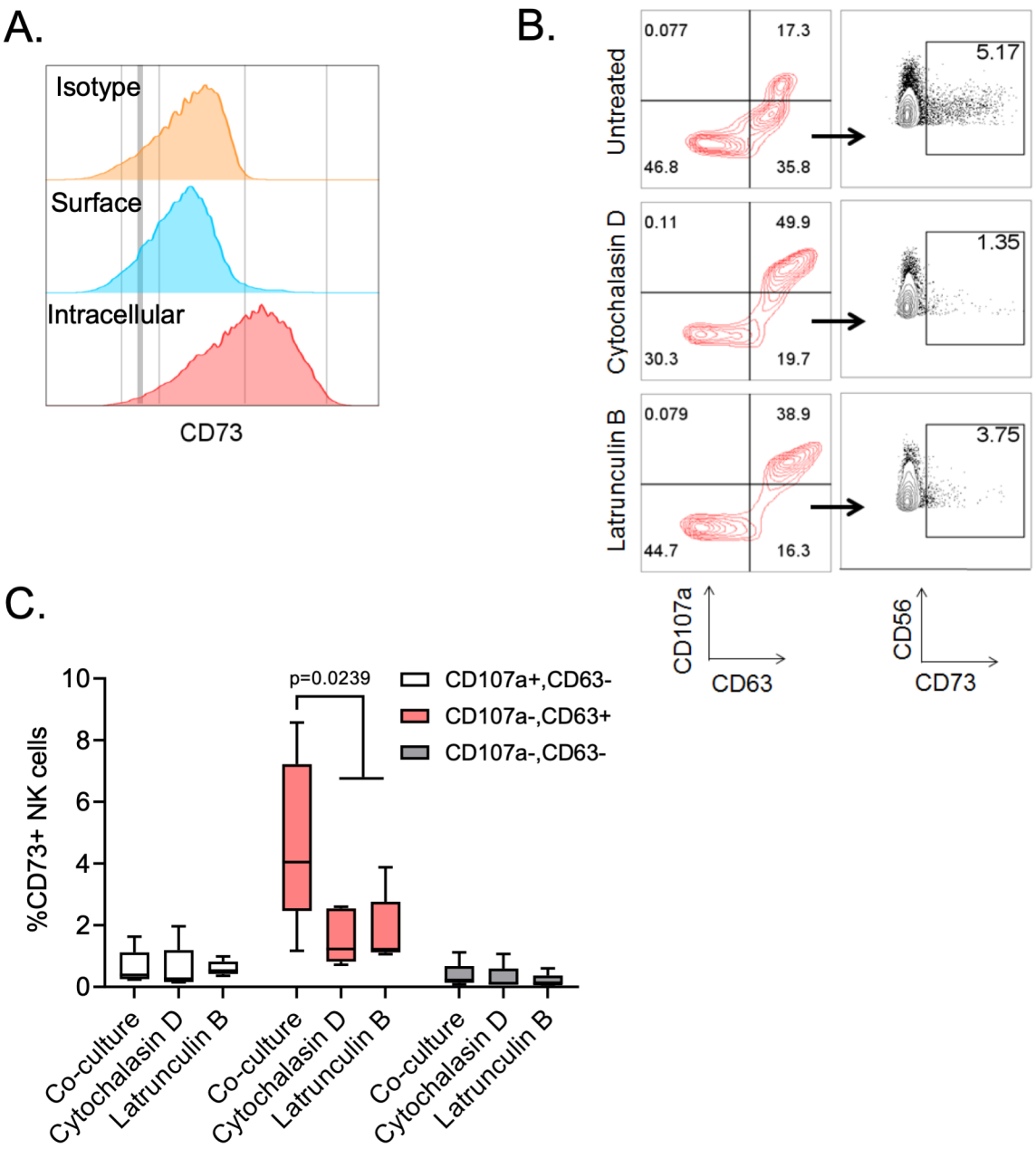
Supplementary Figure 2



Supplementary figure 2.

Tumor-infiltrating CD73+ NK cells express a range of immune checkpoints and activating receptors **A,** Flow cytometric gating strategy performed on samples from tumor resections. **B**, Proportions of attributing parameters used in PCA analysis. (n=11) **C,** Differential expression of immune checkpoints presented in fold change with reference to CD73- NK cells from tumor resections. (n=11) **D to G,** Differential expression of activation receptors (NKG2C, NKG2D, NKp44 and NKp46) comparing CD73+ and CD73- NK cells isolated from tumor resections. Data was collected from 6 tumor resections for figures C, E and F while sample size is 11 for figure D. Wilcoxon signed rank test was done to test for significance in matching data points.

Supplementary Figure 3

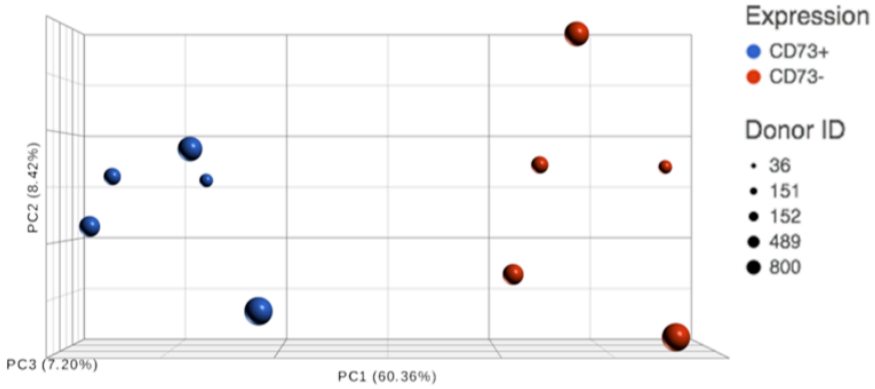


Supplementary figure 3.

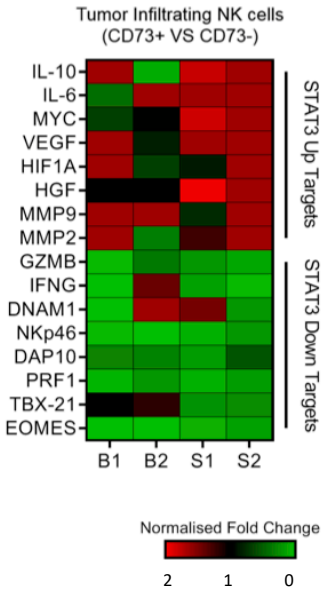
CD73 surface expression is acquired due to vesicular transport and not degranulation. (n=3) **A,** Representative histogram from FACS analysis showing CD73 MFI comparing surface and intracellular staining of IL-2 activated NK cells. **B,** Representative flow cytometric plot showing CD73 expressing cells in CD107a negative and CD63 positive cell populations in the presence of Cytochalasin D (1μM) or Latrunculin B (1μM). **C,** CD73 surface expression on NK cells with or without CD63 and CD107a surface expression in the presence of either Latrunculin B (1μM) or Cytochalasin D(1μM). Friedman test was used to test for significance. (n=4)

Supplementary Figure 4

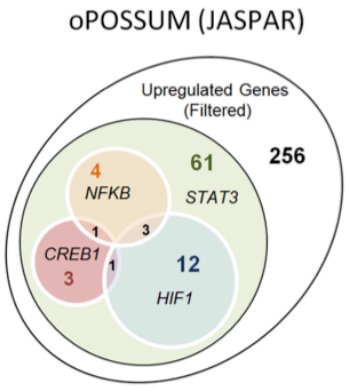
A.



B.



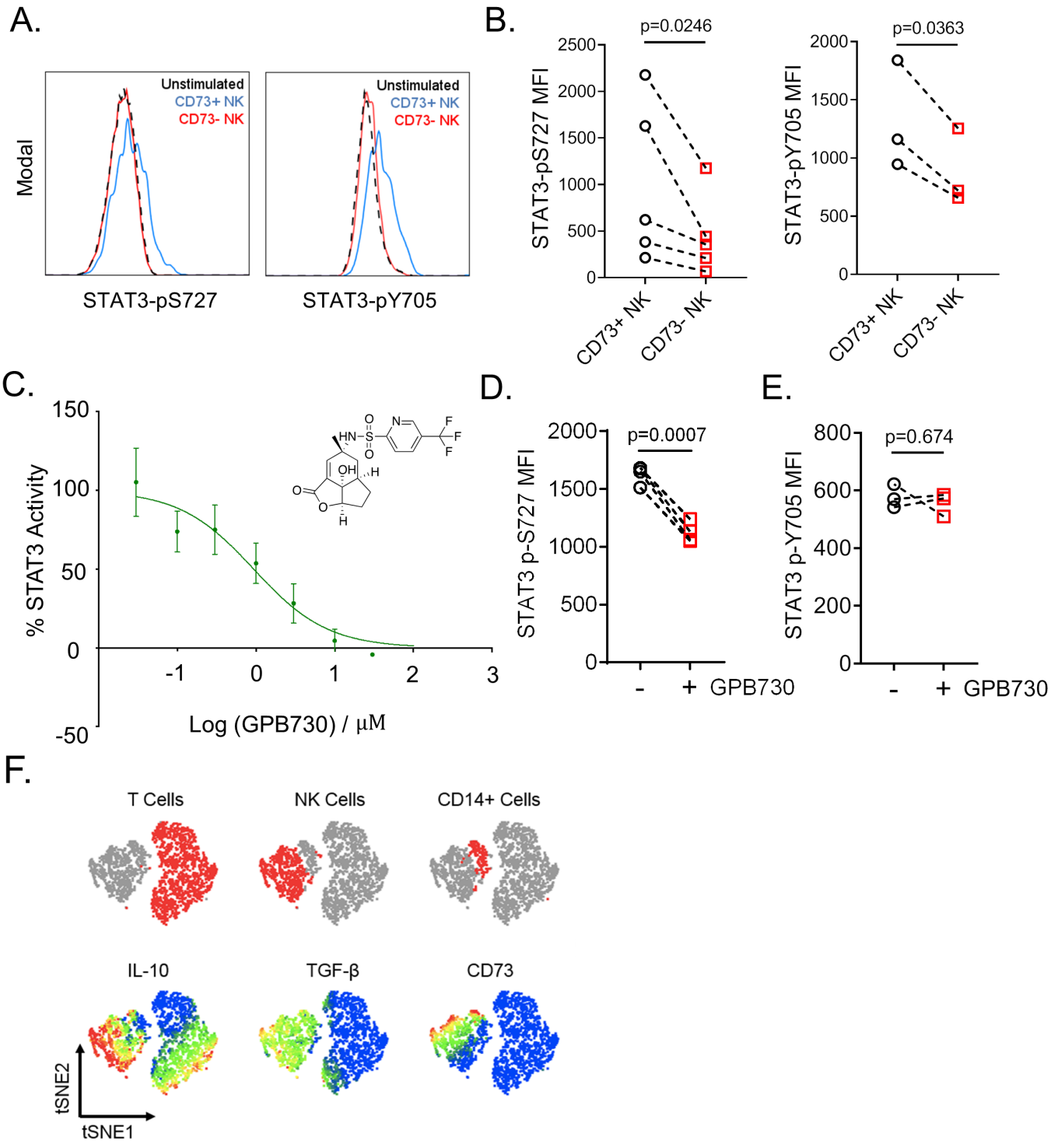
C.



Supplementary figure 4.

Characterization of CD73+ NK cells based on RNA sequencing. **A,** Three-dimensional principal component analysis comparing CD73+ NK cells versus CD73- NK cells from 5 different tumor co-cultures based on differential gene expression. (n=5) **B,** Heatmap showing differential expression of known STAT3-targeted genes comparing CD73+ and CD73- tumor infiltrating NK cells. (n=4) **C,** Venn diagram showing 61 out of 256 upregulated genes with binding motifs for STAT3 based on oPOSSUM-3 platform to query JASPAR database. 259 Genes from supplementary table S2 were used for the analysis with 256 genes recognized by the platform. (n=5)

Supplementary Figure 5



Supplementary figure 5.

GPB730 inhibits phosphorylation of S727-STAT3 in NK cells after 4 hours of tumor co-culture. **A,** Representative histograms showing MFI of phosphorylated STAT3 on S727 and Y705 comparing CD73+ and CD73- NK cells. (n=3) **B,** MFI fold change in phosphorylated STAT3 staining comparing CD73+NK vs CD73-NK. (n=5 for phosphorylated S727; n=3 for phosphorylated Y707) **C,** Chemical Structure of GPB730 and dose-dependent inhibition of STAT3 activity in STAT3 reporter/HEK293 cell line pretreated with GPB730 prior to stimulation with IL-6 for 16 hours. (n=3) **D,** MFI of phosphorylated STAT3 (S727) on NK cells with 10μM of GPB730 during 4 hours of co-culture with K562 transduced with 4-1BBL. (n=3) **E,** MFI of phosphorylated STAT3 (Y705) on NK cells with 10μM of GPB730 during 4 hours of co-culture with K562 transduced with 4-1BBL. (n=3) Paired t-test was used to test for significance. **F,** Representative t-SNE analysis of TILs producing IFN-γ and TGF-β in breast tumors. (n=3)

Supplementary Table S1: List of 8456 differentially expressed genes comparing CD73+ versus CD73- NK cells (Excel File)

Supplementary Table S2: List of 524 differentially expressed genes comparing CD73+ versus CD73- NK cells filtered based on p value<0.05 and fold change>±2.0 (Excel File)

Supplementary Table S3: List of antibodies used for flow cytometry and functional assays (Excel File)