



### Background

TIGIT (T cell immunoreceptor with Ig and ITIM domains) is a recently identified immune-receptor that is expressed on T cells, natural killer (NK) cells and NKT cells. TIGIT has emerged as an important coinhibitory receptor. TIGIT expression on CD8+ tumor infiltrating lymphocytes (TILs) has been shown to be upregulated in solid cancers such as melanoma, colon cancer, and NSCLC and have been associated with a dysfunctional phenotype in TILs. TIGIT can also be activated on a subset of Regulatory T cells (Tregs) and may be critical in driving CD8+ T cell dysfunction. A second mechanism in which TIGIT inhibits immunosurveillance is through both competition and direct inhibition of CD226, impairing its ability to activate immunosurveillance. TIGIT has also been reported to inhibit T cell responses indirectly by triggering CD155 expression in dendritic cells (DCs), thereby preventing DC maturation. These findings render the TIGIT pathway as an attractive candidate for cancer immunotherapy.

In this study, we seek to use MultiOmyx hyperplexed immunofluorescence (IF) assay to exploit this new pathway and characterize the TIGIT expression in a total of 17 melanoma and NSCLC samples. The cancer FFPE slides were stained with a 13-marker panel including TIGIT, CD226, CD155, CD3, CD4, CD8, FOXP3, CD56, CD45, CD11b, CD11c, PD-1, LAG-3, TIM-3 and PanCK. This panel enabled the detection of TIGIT, CD226 and CD115 expression in the melanoma and NSCLC samples. The TIGIT expression was further characterized on different TILs including CD4+ helper T cells, CD8+ cytotoxic T cells, FOXP3+ Tregs and CD56+ NK cells. Using the MultiOmyx proprietary algorithm, we can quantified different subtypes of TIGIT expressing cells and measured the distance of different TIGIT expressing cells to the tumor.

Increased expression of TIGIT and PD-1 has been demonstrated in NSCLC and melanoma. Moreover, TIGIT+ Tregs have been reported to upregulate TIM-3 expression in mice tumor models. This study evaluated the expression of TIGIT in conjunction with other coinhibitory receptors such as PD-1, LAG-3 and TIM-3. The population of TILs that co-express TIGIT/PD-1, TIGIT/LAG-3 and TIGIT/TIM-3 were quantified and analyzed.

Leveraging TIGIT in combination with other immune therapy may achieve more robust clinical outcomes. There are currently multiple phase I clinical trials using TIGIT monoclonal in combination with anti PD-1/PD-L1 antibodies in solid tumors. Our data can help provide more insight into how TIGIT modulate antitumor immunity in melanoma and NSCLC samples. And the findings in this study can also be used to understand the synergistic effects between TIGIT and other coinhibitory receptors and help identify the additional opportunity for combination immunotherapy using checkpoint inhibitors.

## **Overview of Assay Workflow**

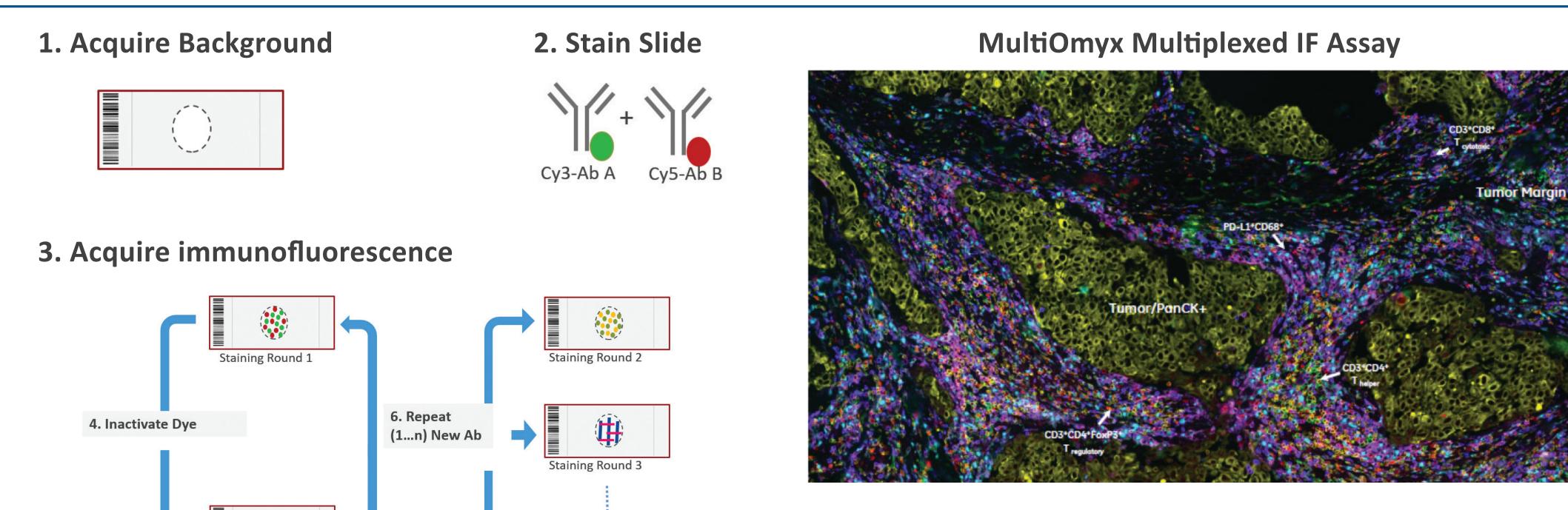


Figure 1. MultiOmyx<sup>™</sup> Assay Workflow. For MultiOmyx IF study, slides were prepared and stained using MultiOmyx multiplexing IF staining protocol. For each round of staining, conjugated fluorescent antibodies were applied to the slide, followed by imaging acquisition of stained slides. The dye was erased, enabling a second round of staining with another pair of fluorescent antibodies.

#### Conclusion

5. Acquire New Backgroun

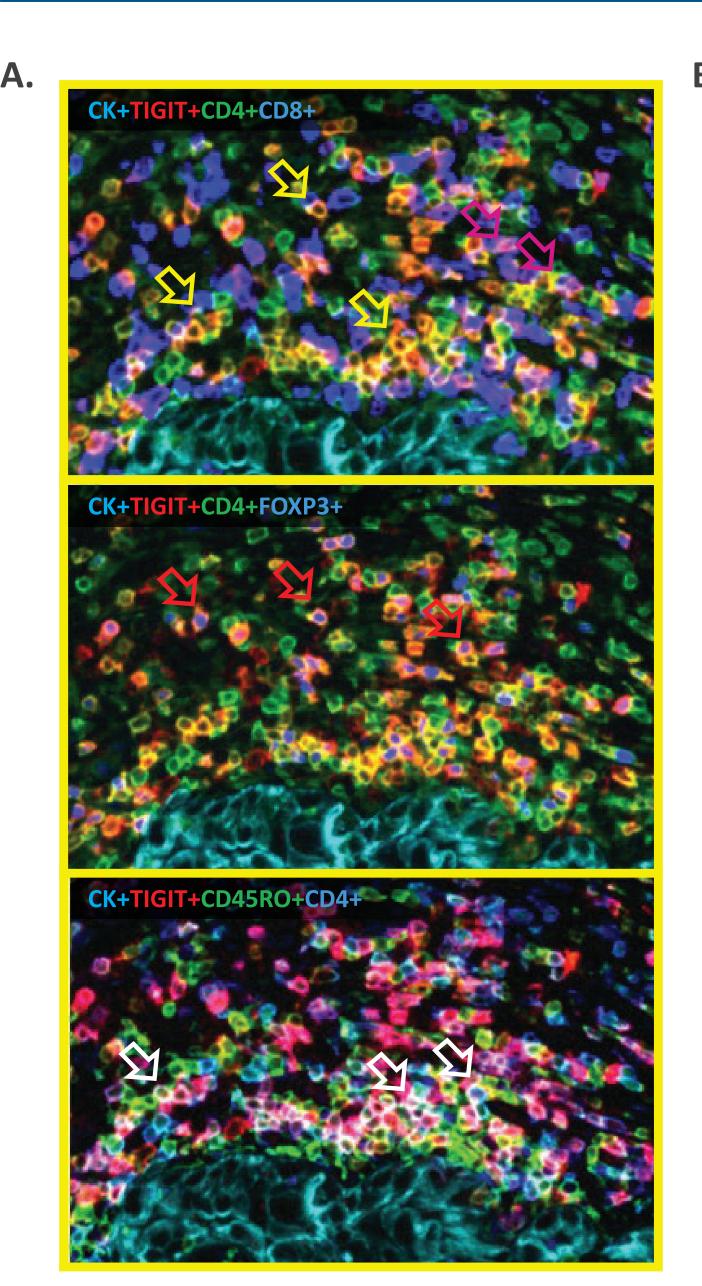
In this study, MultiOmyx technology, a platforms offered by NeoGenomics Laboratories, was utilized to analyze TIGIT expression in different subtypes of lymphocytes and in combination with other immune checkpoint inhibitors in NSCLC and melanoma. • TIGIT was expressed most in T cells including T helper cells, T cytotoxic cells and Tregs and a small subset of NK cells in 7 out of 9

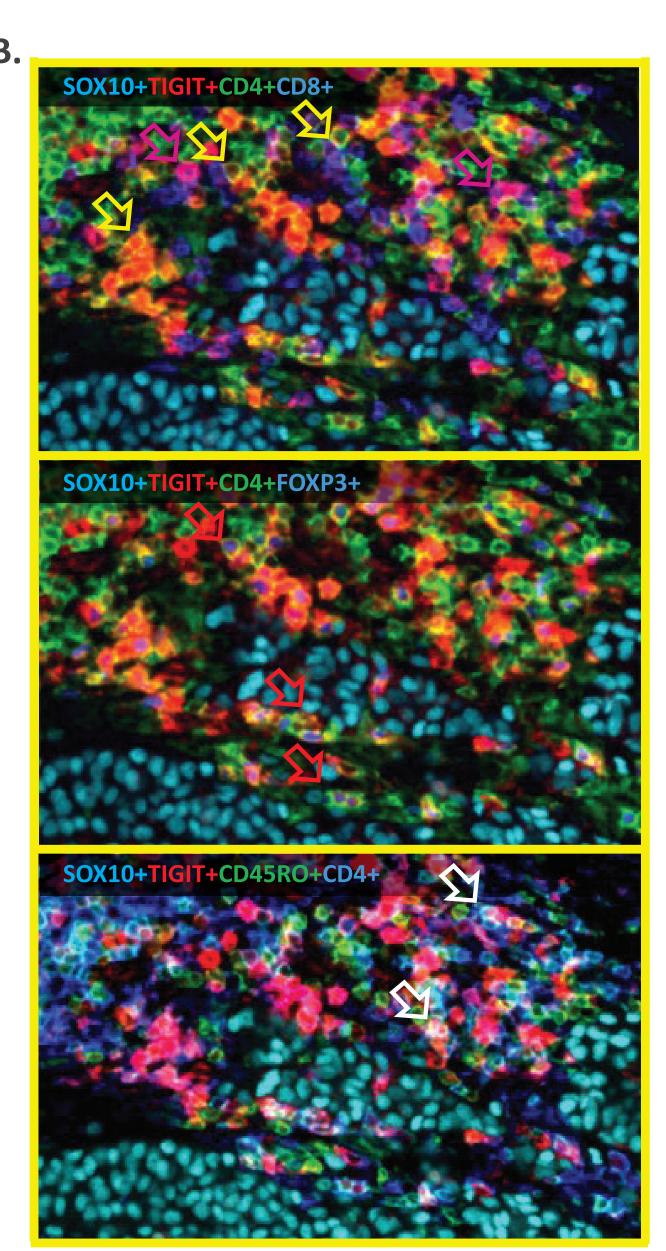
- NSCLC and 6 out of 8 melanoma samples in this study.
- Co-expression of TIGIT and PD-1 was observed in all the TIGIT expressing tumor specimens.
- TIGIT+LAG-3+ and TIGIT+TIM-3+ expressing cells were also present, less abundant than TIGIT+PD-1+.
- Nearest neighbor analysis showed that TIGIT expressing T cytotoxic cells and Tregs are spatially closer to tumor cells than TIGIT expressing T helper cells in both NSCLC and melanoma samples.

# **Characterization of TIGIT Expression Using MultiOmyx™ Hyperplexed** Immunofluorescence Assay in NSCLC and melanoma

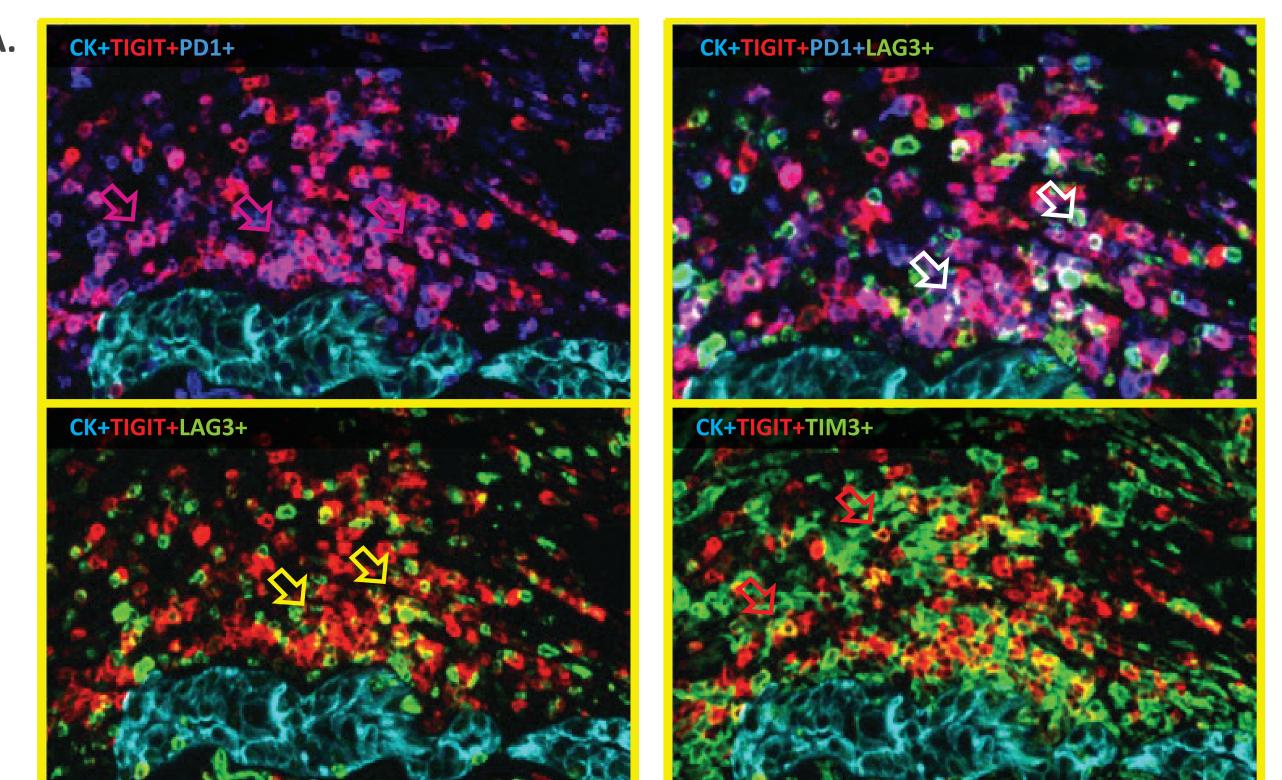
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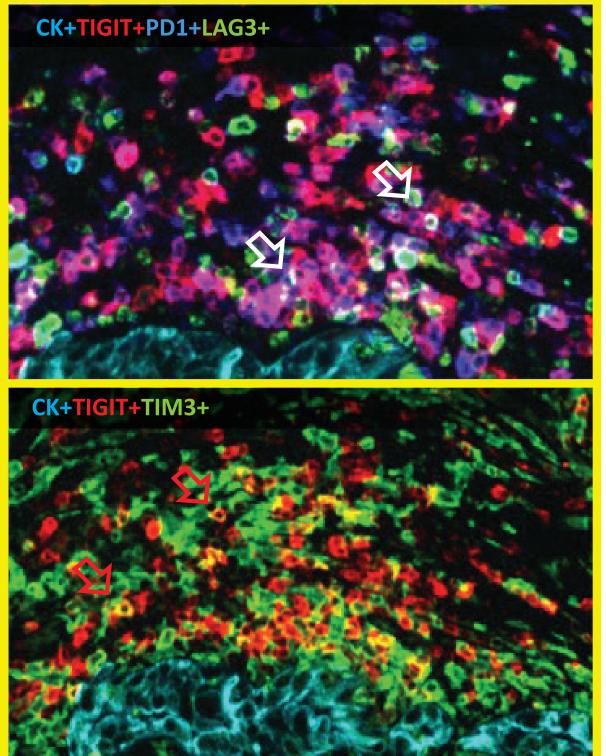
#### **Expression of TIGIT in Different Subtypes of Lymphocytes**

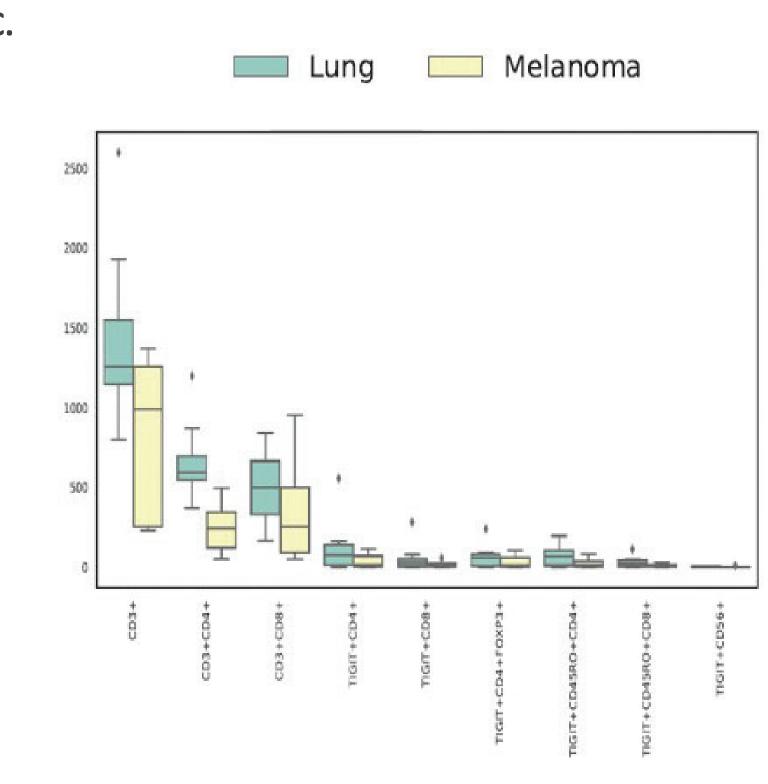




### **Co-expression Analysis of TIGIT with Immune Checkpoint Inhibitors**



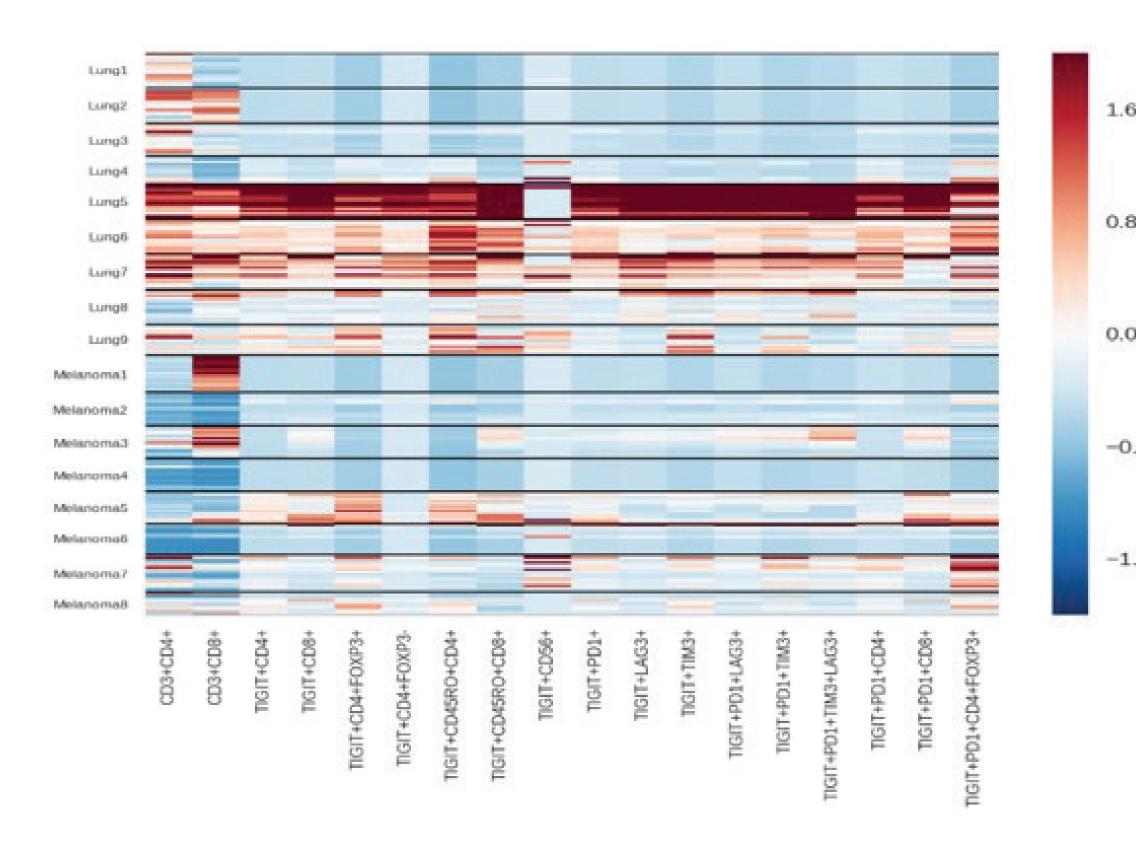


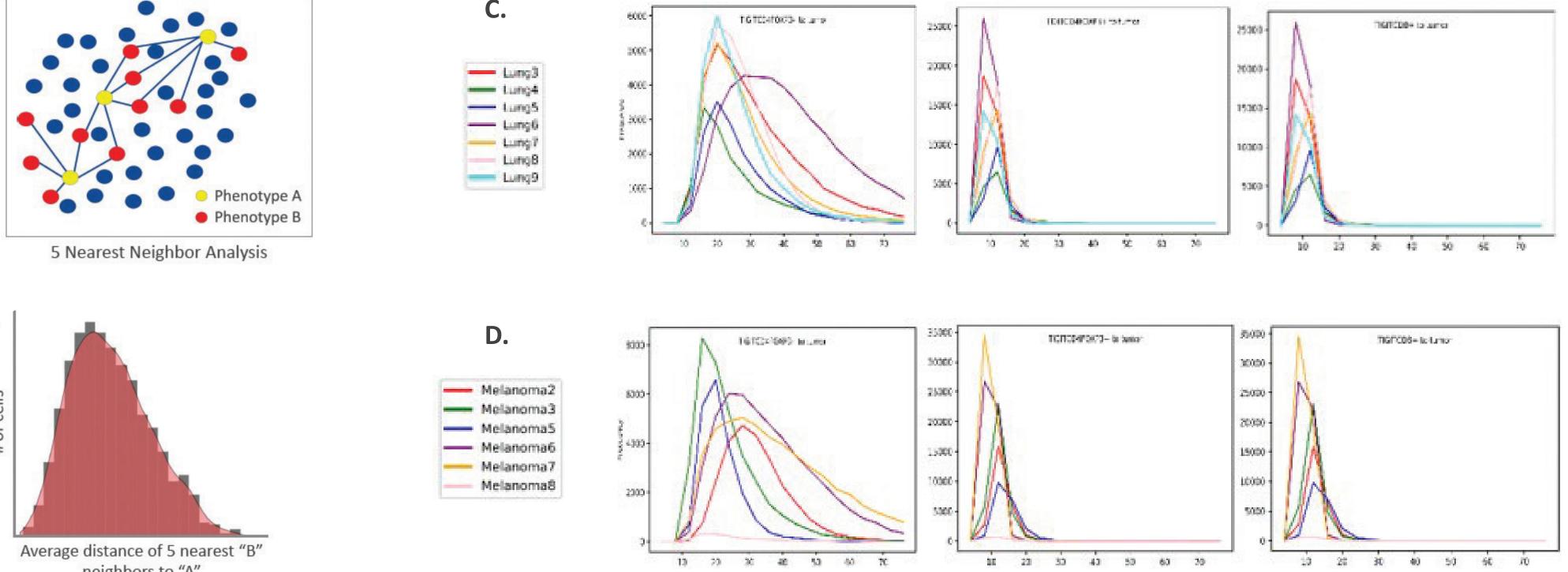


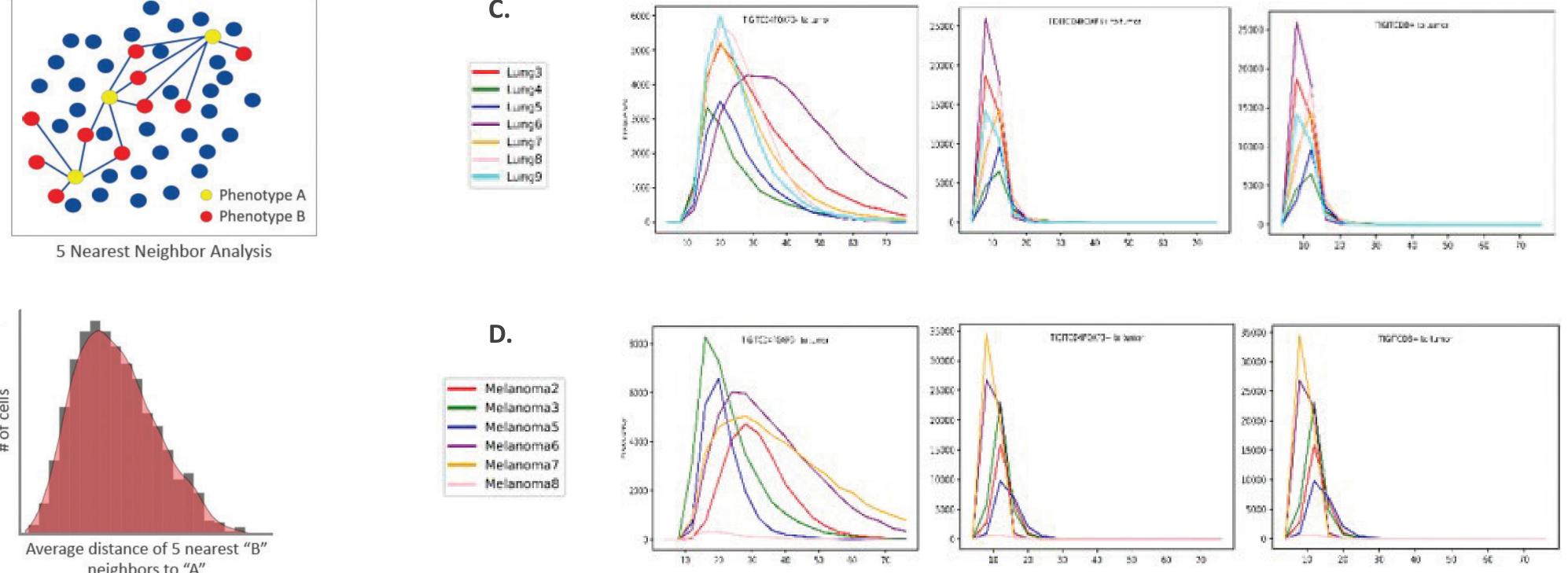
#### Figure 2 Characterization of TIGIT expression in different subtypes of T cells in NSCLC and Melanoma specimens.

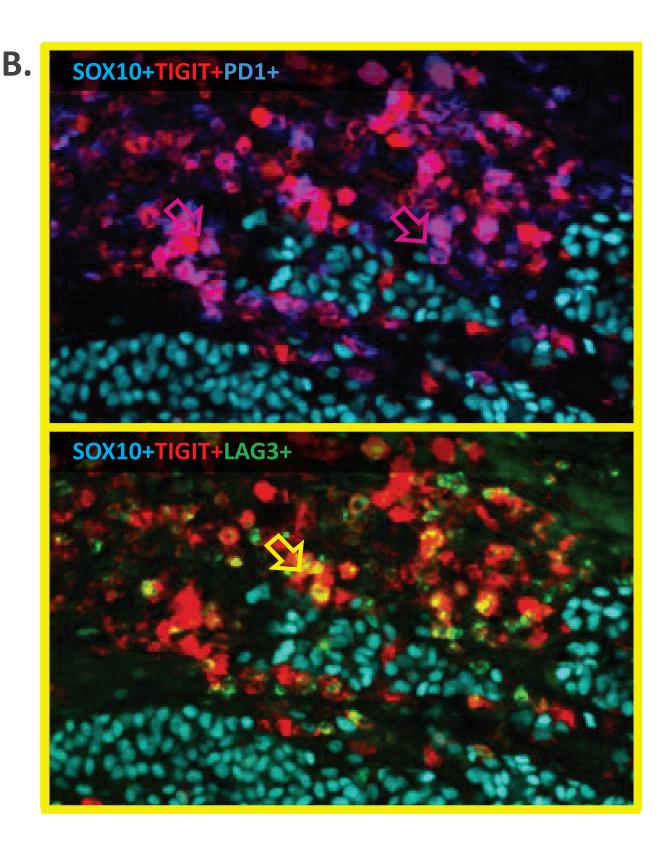
Representative color overlaid images showing co-expression of TIGIT in T helper cells, T cytotoxic cells, Treg and memory T cells in NSCLC (Fig. A) and melanoma (Fig. B). Yellow arrows indicate examples of TIGIT expressing T helper cells (TIGIT+CD4+); magenta arrows (TIGIT+CD8+) indicate examples of TIGIT expressing T cytotoxic cells; Examples of TIGIT expressing Tregs (TIGIT+CD4+FOXP3+) are indicated by red arrows and examples of TIGIT expressing memory T helper cells are indicated by white arrows. **C.** Boxplots to compare the densities of TIGIT expressing lymphocytes in NSCLC and melanoma in the study.

## **Cell Classification and Spatial Analysis of TIGIT Expressing T cells**









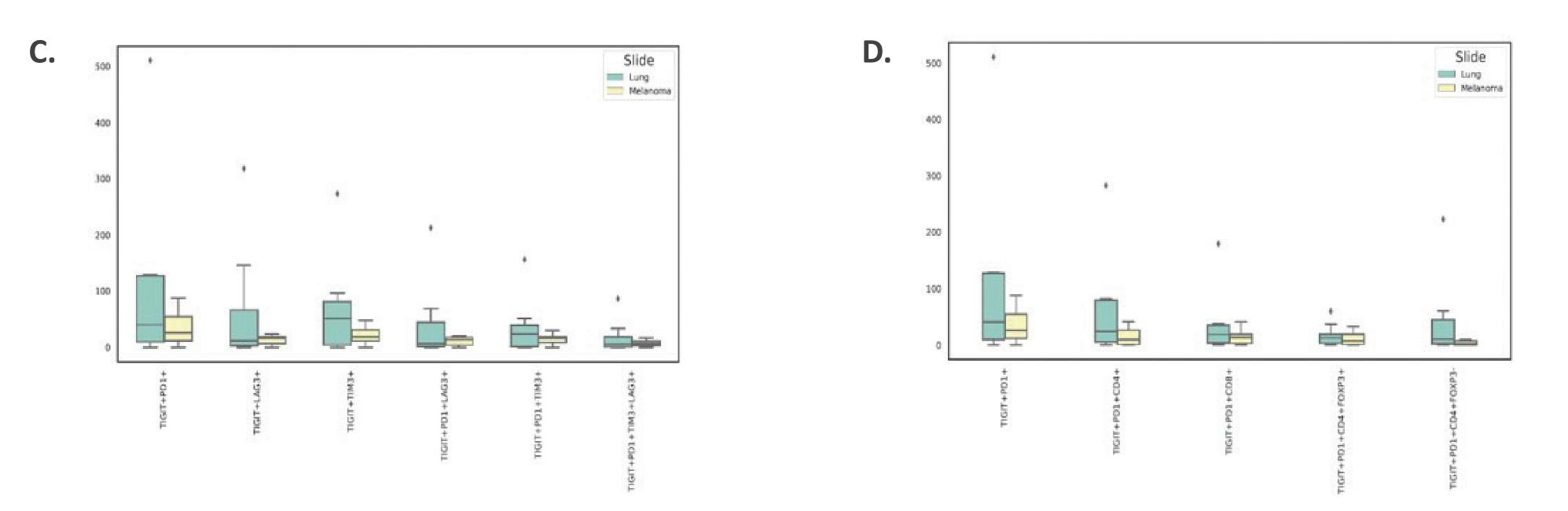


Figure 4 Characterization of TIGIT expression with immune checkpoint inhibitors in NSCLC and Melanoma specimens. Representative color overlaid images showing co-expression of TIGIT with PD-1, LAG-3 and TIM-3 in NSCLC (Fig. A) and melanoma (Fig. B). Magenta arrows indicate examples of cells co-expressing TIGIT and PD-1; yellow arrows indicate examples of cells co-expressing TIGIT and LAG-3; Examples of cells co-expressing TIGIT and TIM-3 are indicated by red arrows and examples of cells co-expressing TIGIT, PD-1 and LAG-3 are indicated by white arrows. C. Boxplots to compare the densities of cells co-expressing TIGIT with the other immune checkpoint inhibitors in NSCLC and melanoma in the study. **D.** TIGIT+PD-1+ co-expression in different subtypes of T cells in NSCLC and melanoma.

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#### Figure 3. Analytics results of TIGIT expression in NSCLC and melanoma specimens.

**A.** Heat map of MultiOmyx TIGIT expressing cell classification results in NSCLC and melanoma. The results are given as densities.

**B.** Nearest neighbor analysis: to calculate the average of the distance of the 5 nearest neighbors from any given phenotypes.

**C.** Spatial correlations of TIGIT expressing T cells to tumor cells in NSCLC samples.

**D.** Spatial correlations of TIGIT expressing T cells to tumor cells in melanoma samples.