High throughput spatial mulitomic assay for assessing immune cell phenotype and function in the tumor microenvironment

Debia Wakhloo, Julia Yu, Ge-Ah Kim, Sonali Deshpande, **Anushka Dikshit**

Advanced Cell Diagnostics, a Bio-Techne brand, Newark, CA 94587

Introduction

Tumormicroenvironment (TME) is a complex milieu of multiple cell types mainly including tumor cells, immune cells, endothelial cells and stromal cells. Immune cell profile within the TME can determine success of immunotherapies, indicate therapeutic efficacy as well as determine any potential therapeutic toxicity. Spatially interrogating these immune cells is crucial in studying immune-immune and immune-tumor interactions. Characterizing immune cells require assessing cell marker expression using protein detection and activation markers using RNA detection. To address this, we have developed a high throughput RNAscope™ assay on the BOND RX to spatially visualize RNA and protein markers on the same slide.

We utilized the new RNAscope™ Multiomic LS assay that can detect up to 6 RNA and protein targets on the same slide. The TSA-based amplification strategy offers signal boost for both RNA and protein targets. To optimize protein detection, the workflow is completely protease-free there by preserving antigen integrity and tissue morphology. The assay can be customized to include any target RNA and proteins of interest. Here, we demonstrate the use of our pre-conjugated antibody panel which includes CD8, CD4, FoxP3 and PanCK to visualize tumor infiltrating lymphocytes (TILs). We also utilized unconjugated primary antibodies for CD68 and CD163 to detect tumor macrophages in multiple tumor tissues.

Infiltrating Cytotoxic T cell lymphocytes were detected using CD8, *GZMB* and *IFNG* co-expression. Regulatory T cells were detected using CD4, FOXP3 co-expression. PanCK was used as a tumor marker to delineate tumor from stroma. Similarly, tumor associated macrophages were detected using CD163, CD68 , *IL10* and *IL-1B* expression. We were also able to identify distinct M1 and M2 macrophage populations in the tumor samples.

The RNAscope Multiomic LS assay was successfully able to interrogate different subpopulation of immune cells with high quality RNA and protein signal while maintaining tissue morphology. The higher plex capability provides detection of more markers simultaneously , in turn enabling immune phenotype profiling while maintaining the sensitivity and specificity that the RNAscope technology is known for.

Methods

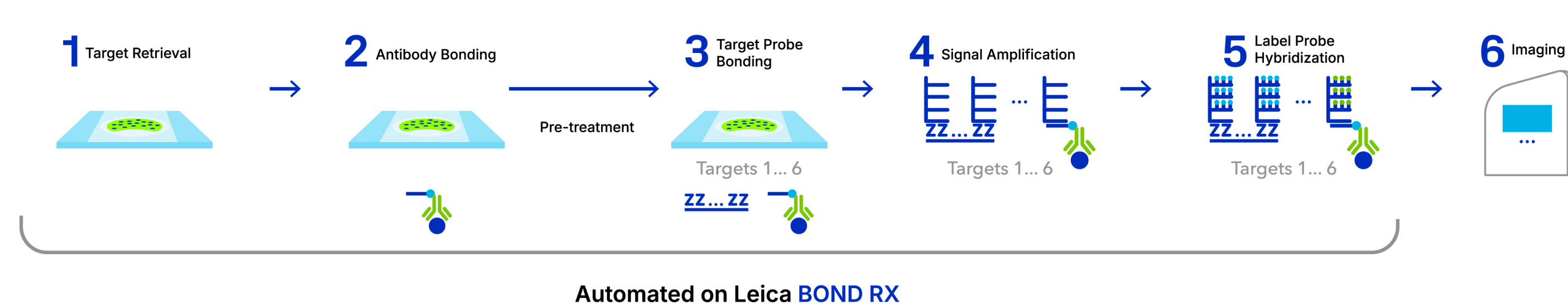RNAscope Multiomic LS assay workflow on the BOND Rx supports 6 plex RNA+protein detection

Figure 1: RNAscope Multiomic LS assay workflow schematic. The new Multiomic LS assay supports detection of 6 plex RNA+protein targets with TSA-based detection. The workflow run on the BOND Rx involves protease-free target retrieval followed by antibody and probe binding , signal amplification and detection.
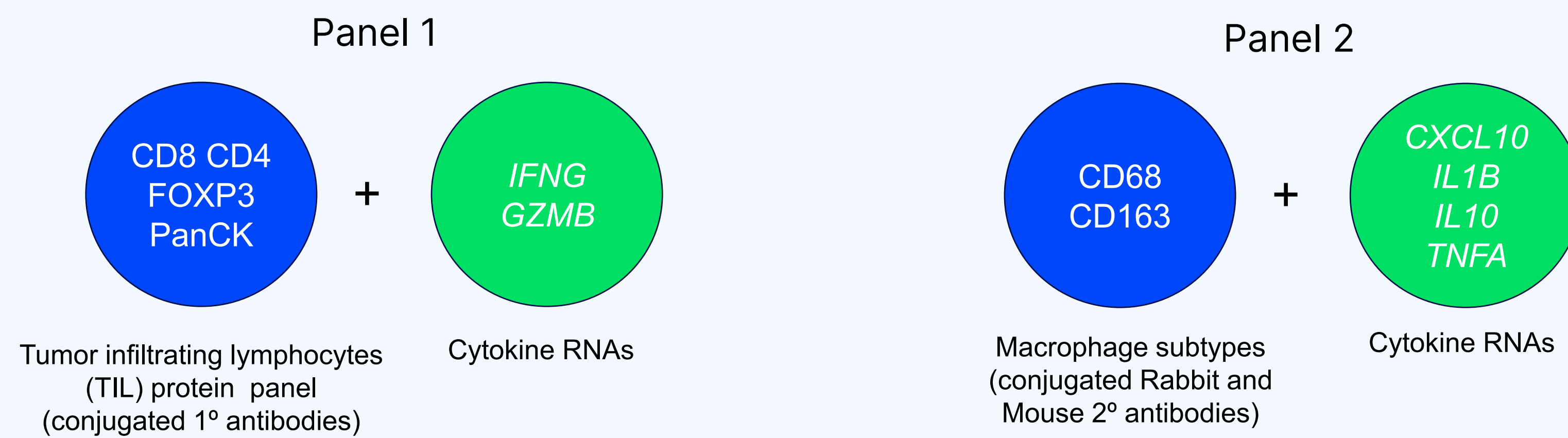
Results

The multiomics TIL panel was able to identify regulatory and cytotoxic T cell populations with cytokine signatures
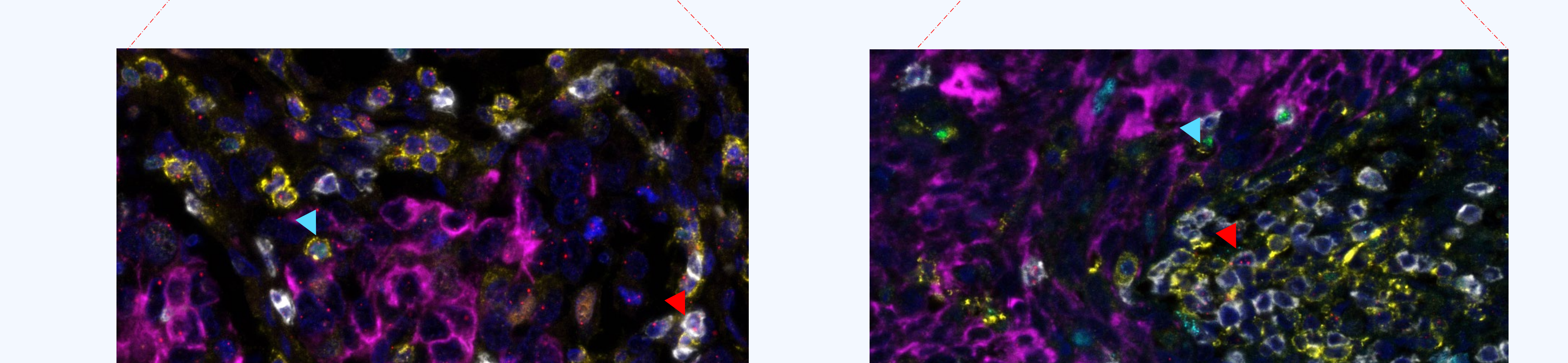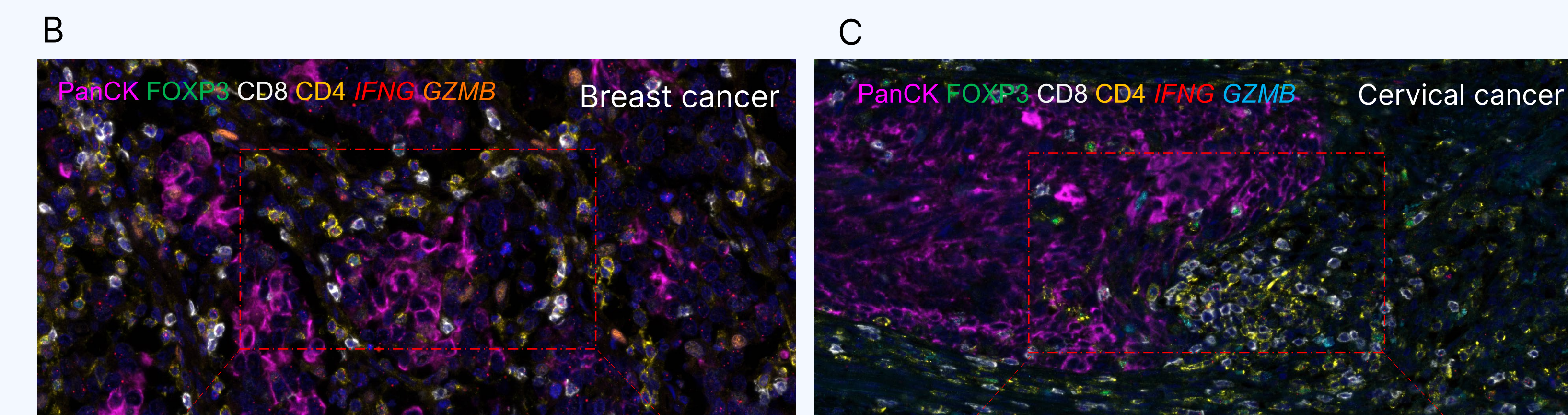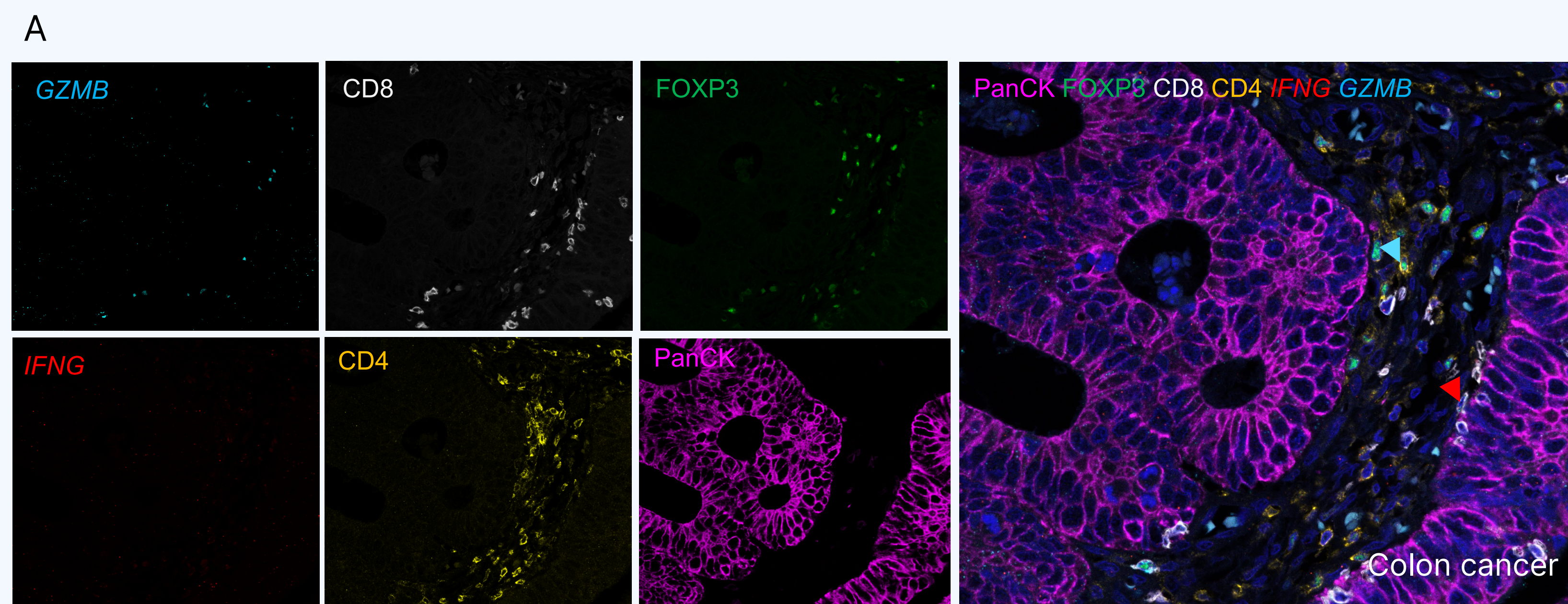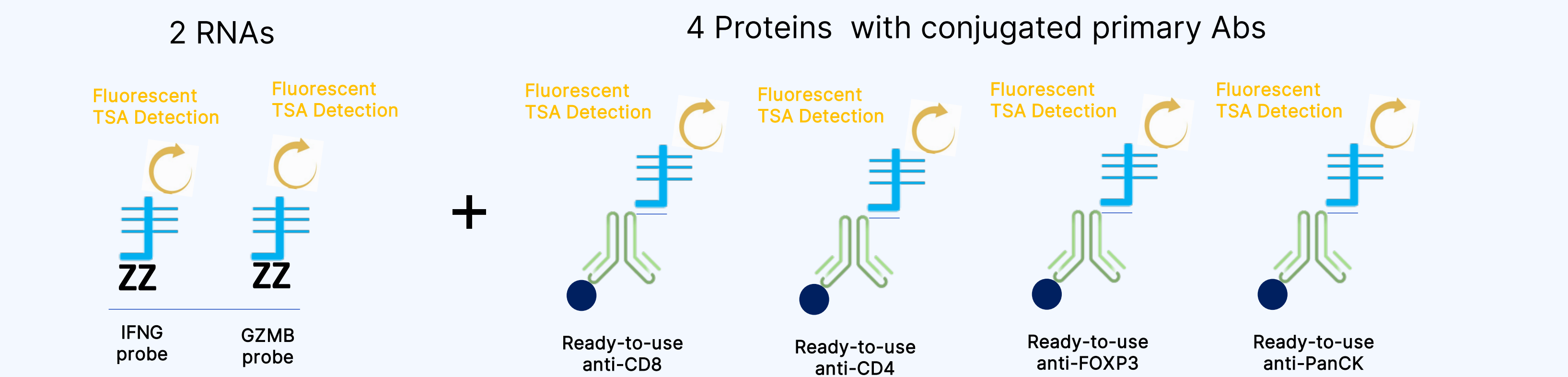

Figure 2. Tumor infiltrating T cells in multiple cancer tissues. Tumor infiltrating regulatory T cells (CD4+/FOXP3+) indicated in cyan arrows and cytotoxic T lymphocytes(CTLs) (CD8+/ *IFNG*+) indicated in red arrows in A, colon cancer, showing CTLs infiltrating the tumor epithelium and B, breast cancer and C, cervical cancer tissues.

Tumor-associated macrophages were identified by detecting co-expression of macrophage-specific marker proteins with cytokine and chemokine RNAs
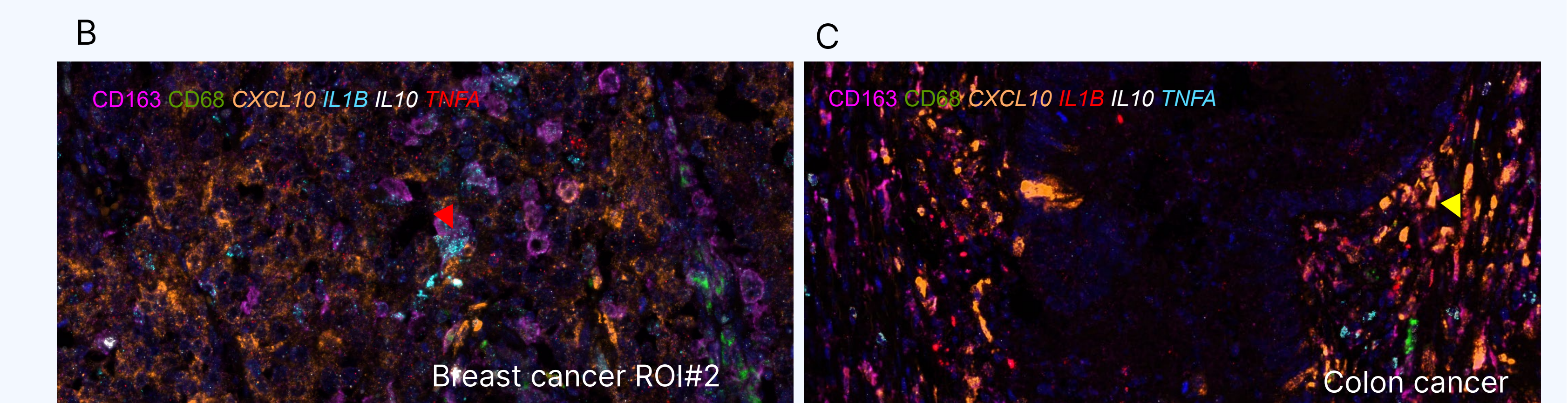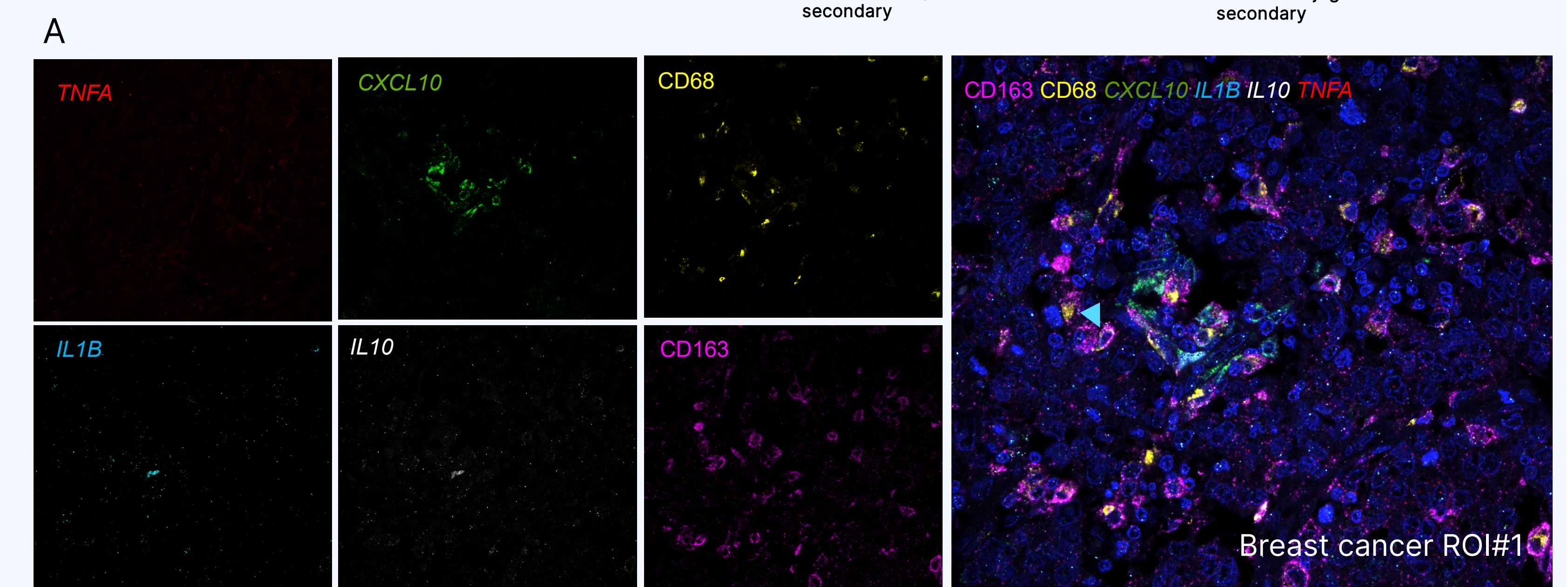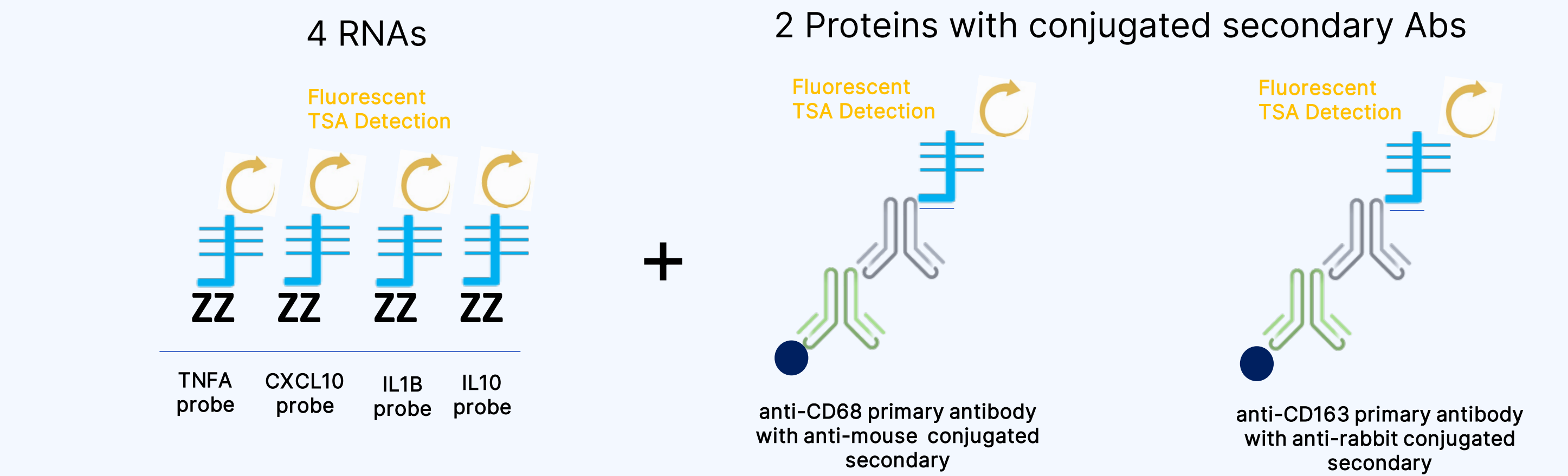

Figure 3. Profiling macrophages in the tumor microenvironment. Cyan arrow indicates tumor associated macrophages (TAMs) (CD163+/CD68+/*IL1B*+/*IL10*+) in A, breast cancer ROI#1, red arrow indicates (CD163+/*IL1B*+) TAMs in B, breast cancer ROI#2 and yellow arrow indicates (CD163+/*CXCL10*+) TAMs in C, colon cancer tissue.

Summary

- With the TIL panel antibodies, T cell subpopulations were successfully visualized along with activating cytokine signatures.
- Using conjugated secondary antibodies macrophage markers such as CD68 and CD163 were used to detect tumor-associated macrophages with secreted cytokine and chemokine RNAs.
- Overall, the RNAscope Multiomic LS assay on the BOND Rx provides the flexibility to combine RNA and protein markers of choice to interrogate the tumor microenvironment.

bio•techne® // Global Developer, Manufacturer, and Supplier of High-Quality Reagents, Analytical Instruments, and Precision Diagnostics.

INCLUDES   R&D Systems™   Novus Biologicals™   Tocris Bioscience™   ProteinSimple™   ACD™   ExosomeDx™   Asuragen®   Lunaphore™