Capturing the Spatial Landscape of Tumor and Immune Cell Lineages in the Microenvironment of Human Cancer Tissues





SITC Poster #1

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Introduction & Aim

Immune system response to cancer therapies can indicate whether a patient will have positive outcomes following therapy. Understanding how the tumor microenvironment (TME) evolves during tumorigenesis and therapeutic response is crucial to developing personalized treatments with the goal of improving cancer therapy. With robust and comprehensive multiplexed imaging technologies, immune biomarkers can be used to interrogate myeloid and lymphoid cell lineages and structures, and when combined with specific oncology biomarkers, can capture the immune response within the TME in a variety of neoplasms. The availability of cell type specific panels, combined with the ability to interrogate using multiplexed tissue imaging, provides unprecedented and novel insights into immune cell populations and spatial cell interactions with many cell types in the TME.

Cell DIVE™ Multiplex Imaging Solution allows probing and imaging of dozens of biomarkers on a whole single tissue section with an iterative staining and dye inactivation workflow. At its core, Cell DIVE is a precise and adaptable open multiplexing solution that enables flexibility in antibody selection of biomarker panels used in a multiplexed imaging study. Cell Signaling Technology (CST) has a broad portfolio of IHC-validated antibodies to detect key proteins in the TME, enabling immune cell detection and phenotyping in tissue. CST offers off the shelf (OTS), ready-to-ship antibody conjugates that have been verified to work on Cell DIVE and offers custom conjugation of IHC-validated antibodies to fluorophores and other detection reagents. CST employs a rigorous approach to IHC validation, followed by verification on the Cell DIVE platform to ensure successful detection of proteins. Here, we demonstrate multiplexed Cell DIVE imaging using a novel panel of dozens of CST biomarker antibodies™ across multiple tissue types. Development of the multiplexed panel required minimal optimization, enabled the identification of complex cell types, and revealed their cell-to-cell interactions within the tumor microenvironment.

Result

Characterizing the tumor microenvironment can help to elucidate new mechanisms that contribute to poor patient outcomes. The tumor microenvironment is complex and commonly heterogenous both within a single sample and across patient samples. Iterative multiplexed staining and imaging enables TME interrogation across tissue samples even in cases where tissue availability is limited. In this study, we have examined the expression of more than 30 biomarkers across 12 full tissue or TMA slides (Table 1), with a focus on potential immunotherapy targets, predictive biomarkers and segmentation markers. All CST biomarker antibodies were conjugated and randomly assigned to a round, without optimization.

To define immune cells in the context of other cells in the TME, all biomarker images were fused, segmented and expression was analyzed using cluster analysis and dimensional reduction (UMAP). Cluster analysis provides a non-biased approach to define the immune cell contribution within the tissue. In this study, we show cluster analysis for human colon adenocarcinoma (CAC; Fig.1). Here, cluster resolution allows the separation of leukocytes from all other epithelial and stromal cell types (Fig. 1C). Further, clusters clearly define lymphoid and myeloid classes. Myeloid subtypes in CAC include myeloid progenitor, M2 macrophage and two other myeloid clusters of unknown subtypes. Additional biomarkers can be used to further define subtypes. For the lymphoid class, T cell and NKT cell clusters were defined. An additional class of T cell with CD20 positivity was identified. Cell types can be further defined from clusters using machine learning for single cell phenotype analysis. For example, in image 1D, a cell in Cluster 15 is CD45+ CD3+ CD8+ CD4+ GRZB+ Ki67+ LAG3+ PD1+ TIM3+ (Fig. 1D-E; orange circle). Cluster and single cell spatial analysis were applied to all other tissues in the study (Fig. 2; data not shown).

Table 1. Study Design: Antibodies and Tissues

				Conc.	
Cat.#	Antibody	Clone	Conjugate	(ug/mL)	Dil.
9854S	Vimentin	D21H3	AF_488	200	1:100
34105S	SMA	D4K9N	AF_488	125	1:100
4523S	Panck	C11	AF_488	100	1:100
3478S	Panck	C11	AF_555	32	1:100
23308S	CD68	D4B9C	AF_555	4.5	1:100
*	SMA	1A4	AF_488	100	1:100
*	Sox9	D8G8H	AF_488	100	1:100
*	ATP1A1	D4Y7E	AF_488	100	1:100
*	CD79A	D1X5C	AF_555	100	1:100
*	CD3E	D7A6E™	AF_555	100	1:100
*	CD20	E7B7T	AF_647	100	1:100
*	CD163	D6U1J	AF_647	100	1:100
*	Ki-67	D2H10	AF_647	100	1:100
*	CD8α	D8A8Y	AF_750	100	1:100
*	CD45	D9M8I	AF_750	100	1:100
*	PD-1	D4W2J	AF_750	100	1:100
*	Vimentin	D21H3	AF_750	100	1:100
*	FoxP3	D2W8E™	AF_647	500	1:100
*	CD4	D7D2Z	AF_555	200	1:100
*	CD56	E7X9M	AF_647	200	1:100
*	PDL-1	E1L3N	AF_647	200	1:100
*	CTLA-4	E2V1Z	AF_647	200	1:100
*	CD11B	D6X1N	AF_750	200	1:100
*	LAG3	D2G4O	AF_750	200	1:100
*	GZMB	D6E9W	AF_555	200	1:100
3655	GFAP	GA5	AF_488	50	1:100
78226	TIM-3	D5D5R	AF_647	100	1:100
61255	CD31	89C2	AF_555	47	1:100
5108	EGFR	D38B1	AF_555	50	1:100
7497	pNDRG1	D98G11	AF_647	25	1:100
3906	GAPDH	14c10	AF_488	100	1:100
2866	SURVIVIN	71G4B7	AF_647	50	1:100
5067	SOX2	D6D9	AF_647	25	1:100

Slide	Tissue	Catalog
	Human Metastasis	
1328	TMA	MTU961
1329	Multi-Tumor TMA	MTU951
	Mucinous	
	Cystadenocarcinoma,	
1330	Ovary	OVA13
1331	Melanoma, Anus	SKI04
1332	Multi-Tumor TMA	MNT651
	Ductal	
	Adenocarcinoma,	
1333	Pancreas	PAC02
	Large Cell Carcinoma,	
1334	Lung	LUN17
1335	Multi-Tumor TMA	MTU481
	Chronic Bronchitis,	
1336	Lung	LUN04
	Hepatocellular	
1337	Carcinoma, Liver	LIV05
	Invasive Ductal	
1338	Carcinoma, Breast	BRE06
	Adenocarcinoma,	
1339	Colon	COL04

Custom conjugations provided by CST

Methods & Materials

CST antibodies undergo a vigorous validation process to ensure antibody performance on FFPE tissue. All antibodies in this study were direct conjugates or off the shelf (OTS) commercial conjugates (Table 1). Conjugation was performed using non-site-specific chemistry. Antibodies were conjugated with four different dye molar excesses, unbound dye removed, and the degree of labeling measured by spectrophotometric analysis. Following preliminary validation, conjugated antibody solutions with the optimum degree of labeling and concentration were used for subsequent stainings of a variety of human cancer tissues and normal tissue. Tissue was obtained from a commercial source (Pantomics; Table 1). Slides were imaged on the Cell DIVE using four channels plus DAPI, with automatic AF removal, corrections and stitching. Fully stitched images were imported, fused, and analyzed using proprietary software developed by Leica Microsystems that will be commercially available in the first half of 2023.

Conclusions

- Iterative staining and imaging of a 12 full tissue and TMA slides with more than 30 CST biomarker antibodies using the Cell DIVE multiplexed imaging solution.
- This CST panel enabled the identification of clusters containing cells of different immune classes, cell types and subtypes.
- Cell DIVE multiplexing solution is tissue preserving, future work to further define immune cell subtypes can continue using additional CST antibodies on the same tissue sections, overlayed with all previous biomarkers from the study.

igure 1.

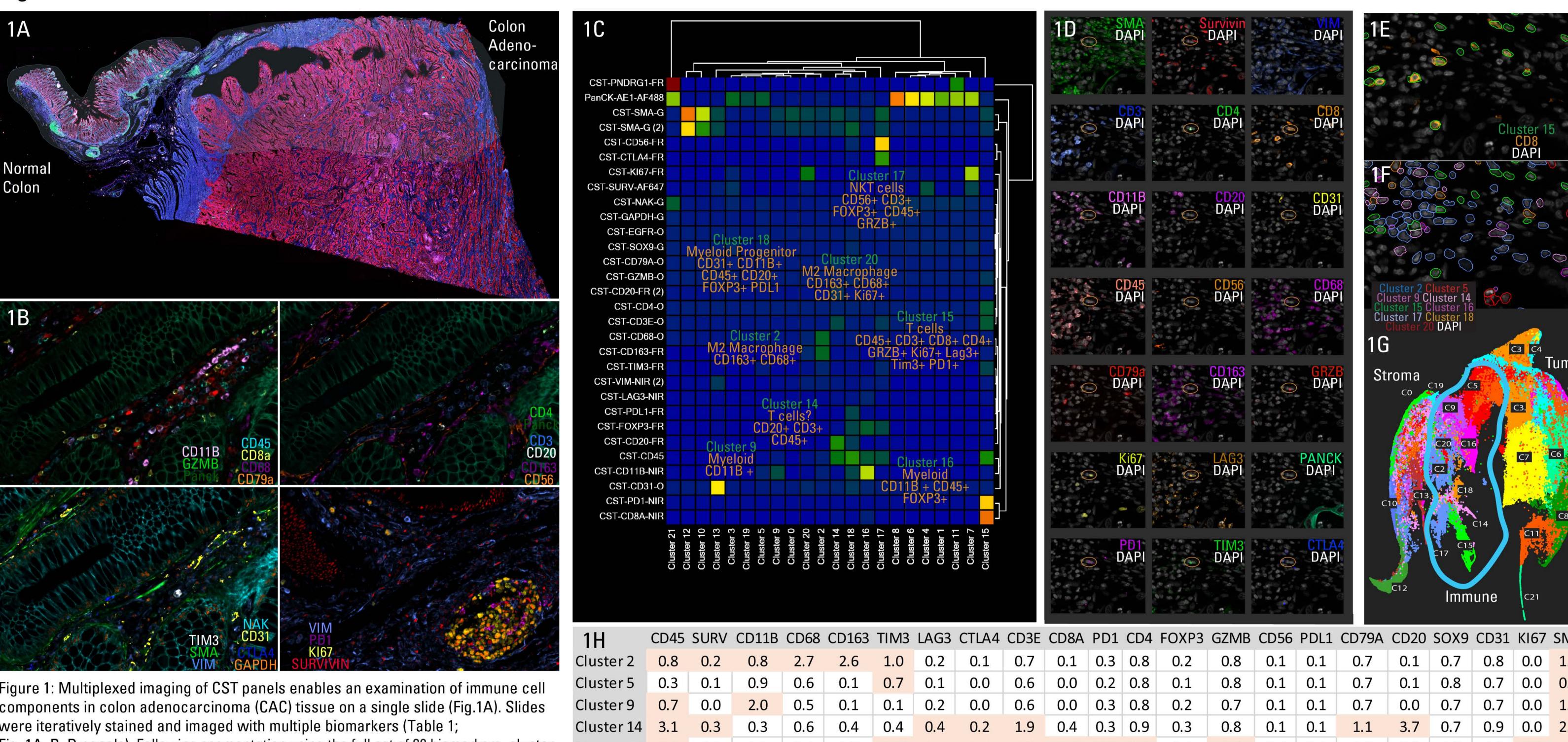


Figure 2.

PanCK is clone AE1/AE3 AF488 conjugate.

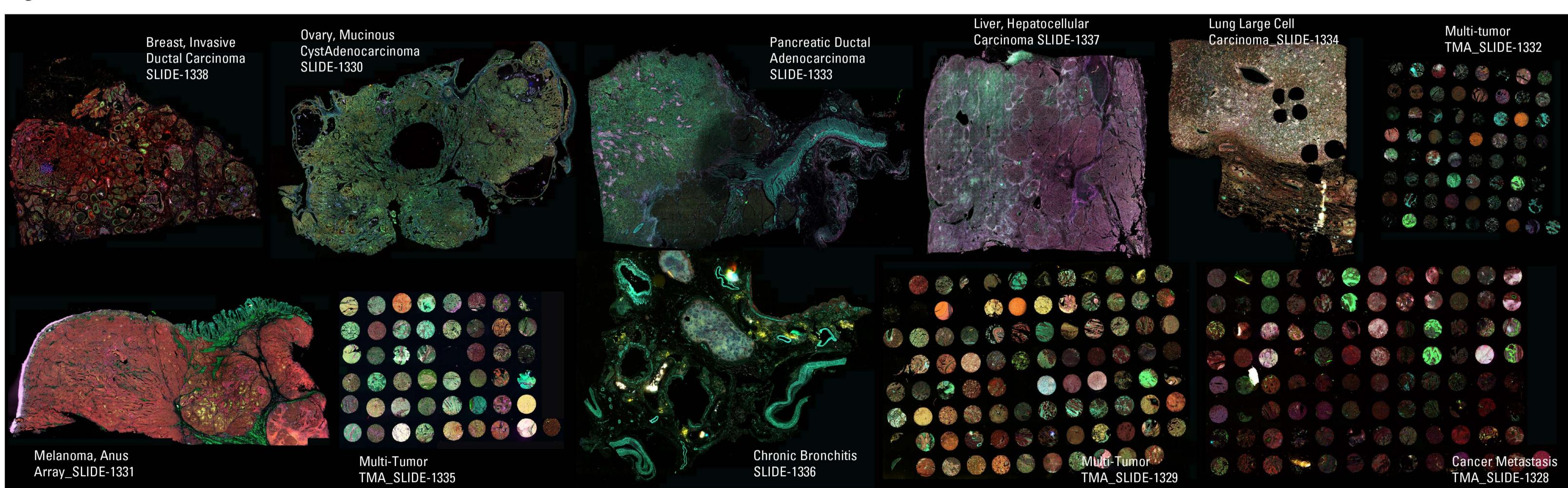


Figure 2: Multiplexed imaging of CST panels in multiple cancer and normal tissue types. Slides were iteratively stained and imaged (Table 1; Fig. 2 tissue panels). Optimal intensity values for all biomarkers are shown in one image. Following segmentation using the full set of 30 biomarkers, cluster analysis revealed immune cells within the tissue (data not shown). Tissue specific immune cell clusters are statistically calculated from specific biomarker expression patterns. Following clustering, single cell phenotyping enables spatial analysis of cell population within the clusters.

Questions?

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s revealed the immune cells within the tissue (CAC shown in 1C-H, normal

tissue not shown). Cluster 15 (Fig. 1D-F) is highlighted and a specific cell

cross biomarkers is highlighted with an orange circle (Fig. 1D). Dimension reduction