

Expandable Immunofluorescence Assays with Next-Generation VistaPlex Immune Profiling Kits

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Introduction

VistaPlex™ assay kits are modular ready-to-use multiplex immunofluorescence (mIF) assays designed to provide in-depth cellular phenotyping and tissue information for spatial biology assays. The CellScape™ Precise Spatial Proteomics platform was recently upgraded to utilize EpicIF™ technology for expanded multiplex dye capabilities. Next-Generation VistaPlex assay kits were created to be compatible with the EpicIF workflow. Here, data demonstrate the utility of Segmentation, Spatial Immune Profiling, and Architecture panels for a broad range of human FFPE tissues. Our data demonstrate unique biological observations that can be revealed with each kit alone or in combination.

Methods

Experiments were performed on the CellScape platform following the new EpicIF workflow upgrade. Following inter- and intra-assay reproducibility and validation experiments, we tested three ready-to-use antibody panels against four different human FFPE tissue types. Downstream segmentation was performed using CellPose on membrane and nuclear markers from the segmentation kit. Cell phenotyping was conducted by unsupervised clustering on the spatial immune and architecture markers. Cellular neighborhoods were calculated using the K-means nearest-neighbors approach.

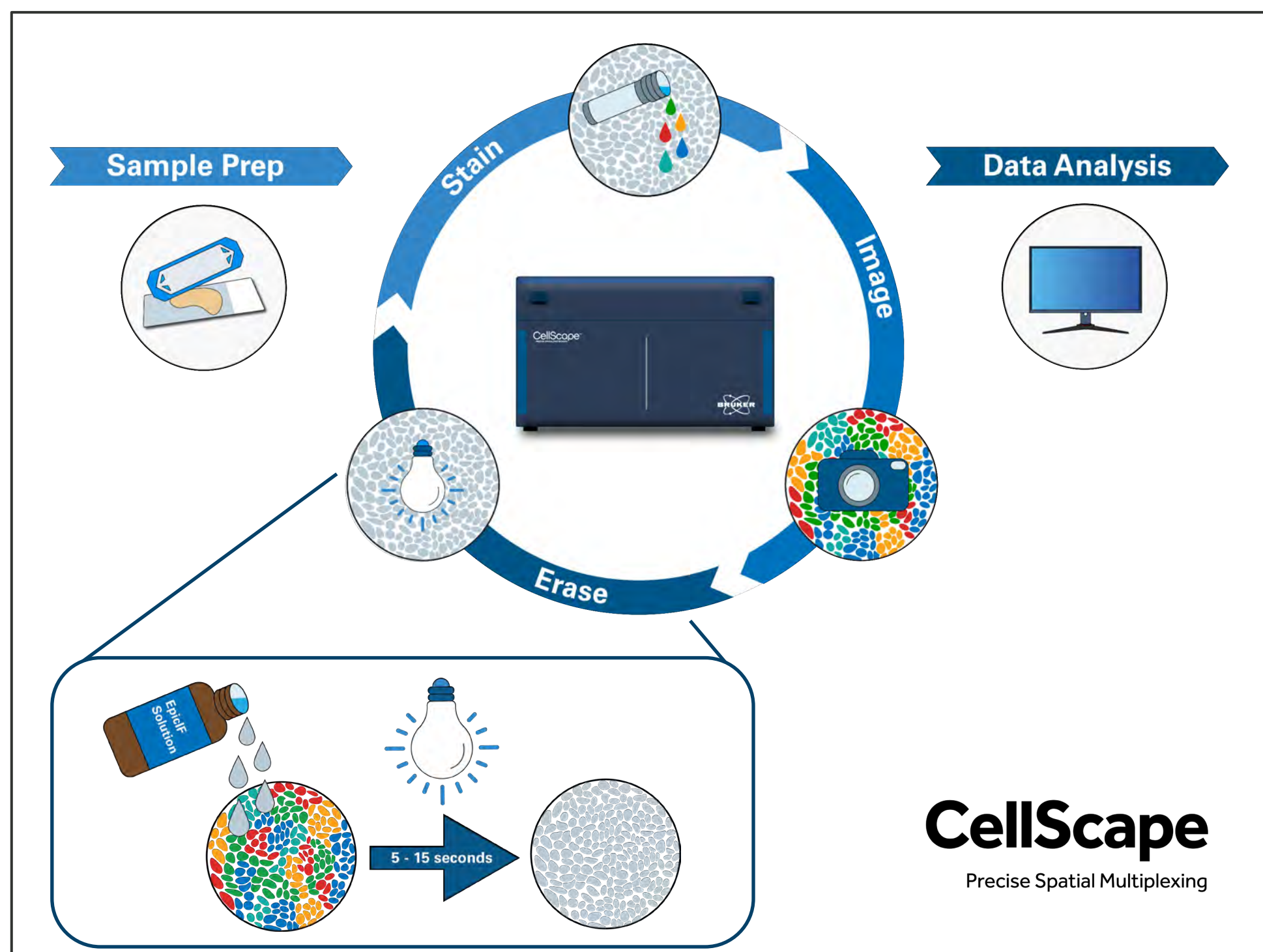


Figure 1. The EpicIF workflow on the CellScape platform. The instrument uses cycles of staining, imaging, and signal removal to detect biomarkers with spatial context at single-cell resolution. Signal removal facilitated by filtered photobleaching and EpicIF™ Solution is safe, gentle, and effective.

1. Segmentation		2. Spatial Immune Profiling		3. Tissue Architecture	
DNA	LaminB1	CD20	GrnB	CD31	Collagen-IV
B2M	CD4	CD56	CD8	CD138	Podoplanin
ATP1A1	CD19	CD3	CD45RO	E-Cad	B-Catenin
		PD-1	CD38	Vimentin	
		PD-L1	Ki67	E-Cad	
		FoxP3	CD68	CD34	
		CD4	CD45RA	MUC1	
		CD45	PanCK	CD105	
		CD19	CD163	EPCAM	

Results

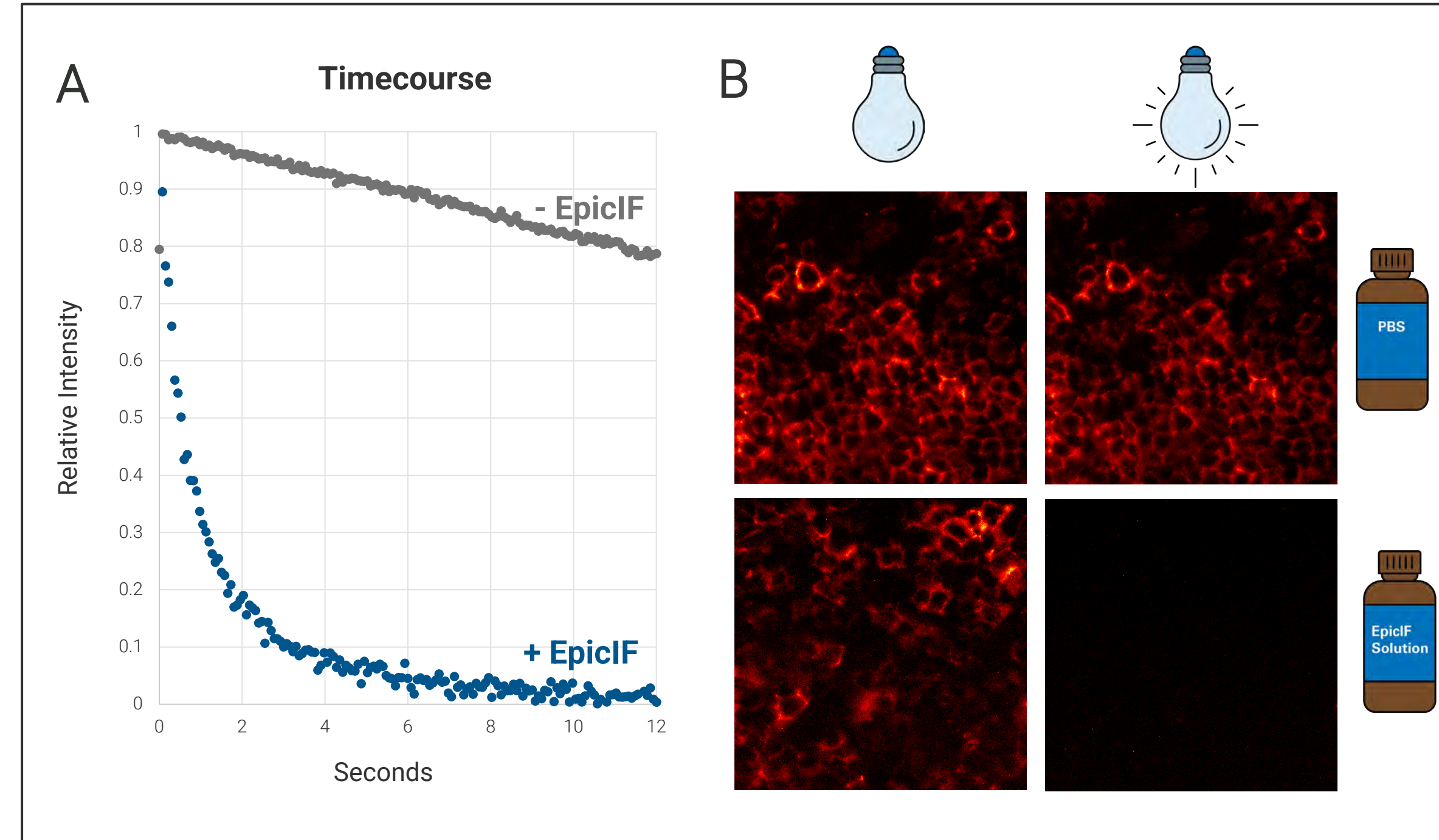


Figure 2. Demonstration of EpicIF efficacy over time. EpicIF effectively removes signal from photostable dyes of the AlexaFluor™ (Thermo) and CoraLite™ (Proteintech) families. A timecourse of CD20-AF647 relative signal intensity with and without EpicIF solution (A) and single position images of CD20-AF647 signal at T0 (light off) and after 5 seconds of photobleaching (light on), with and without EpicIF solution added (B) as an example.

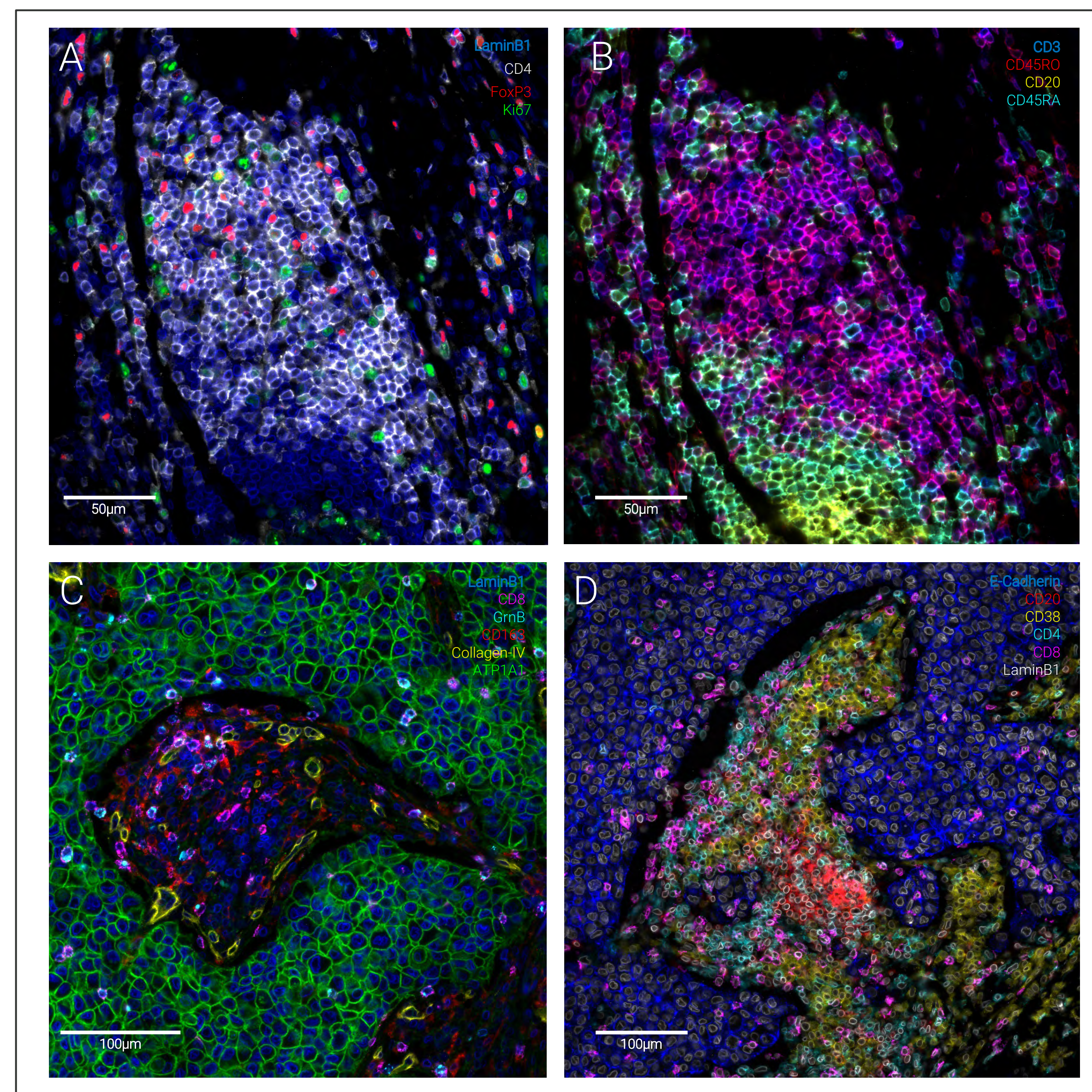


Figure 3. Deep Immune Phenotyping. The Spatial Immune Profiling panel captures complex CD4+ phenotypes with differential FoxP3 and Ki67 statuses (A) and differential CD45RA/CD45RO expression in CD3+ and CD20+ cell populations (B) in human colorectal cancer. Combining the VistaPlex kits enables detection of CD8+ T-cell activation by expression of GrnB (C) and broad immune infiltrate categorization in breast cancer (D).

Figure 4. Human FFPE cancer tissue samples stained with EpicIF-compatible VistaPlex panels. A,D,G, Segmentation panel. B,E,H Spatial Immune Profiling panel. C,F,I, Tissue Architecture panel. Markers are as shown in insets, and cancer types are indicated on the left side.

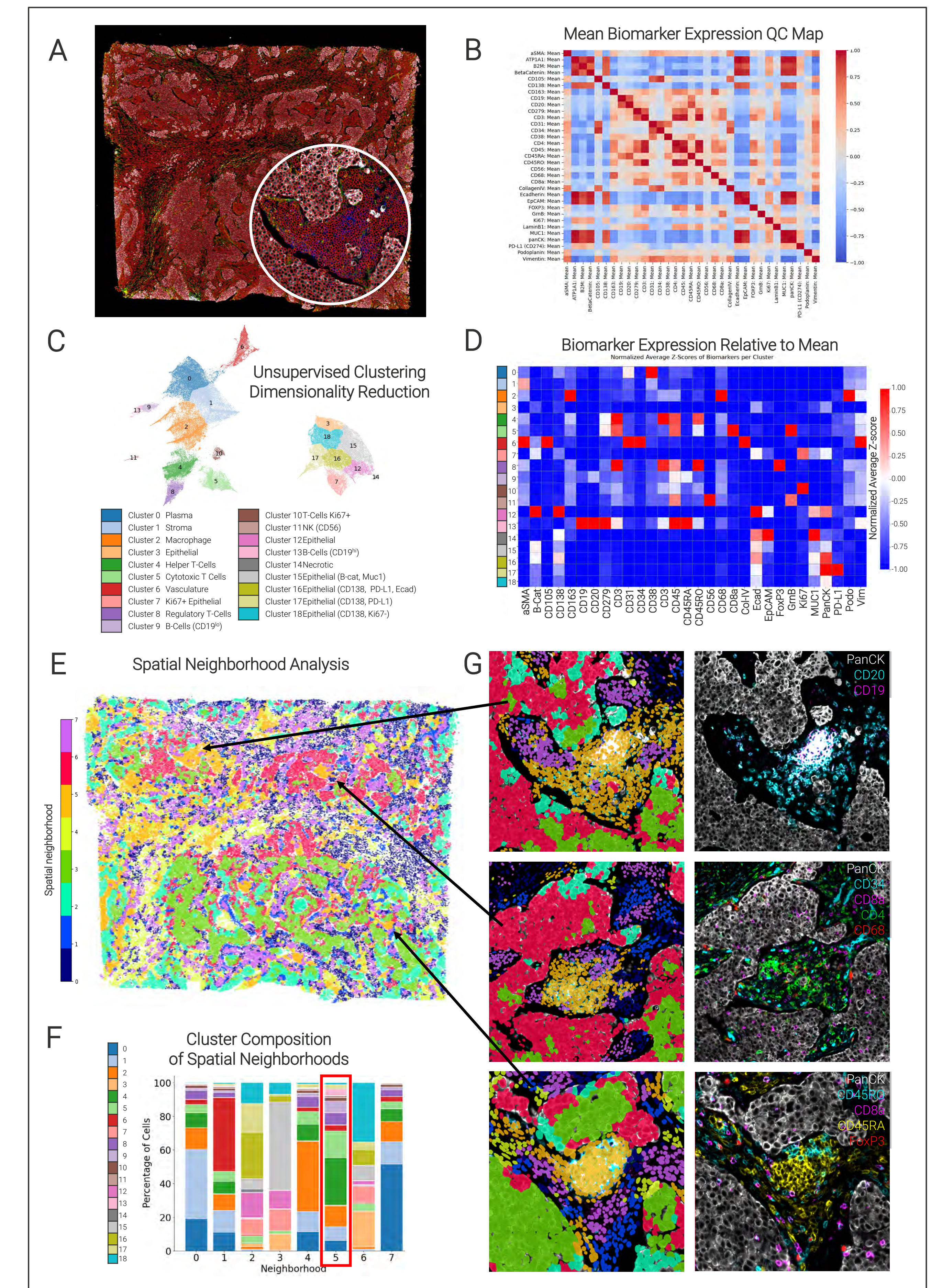
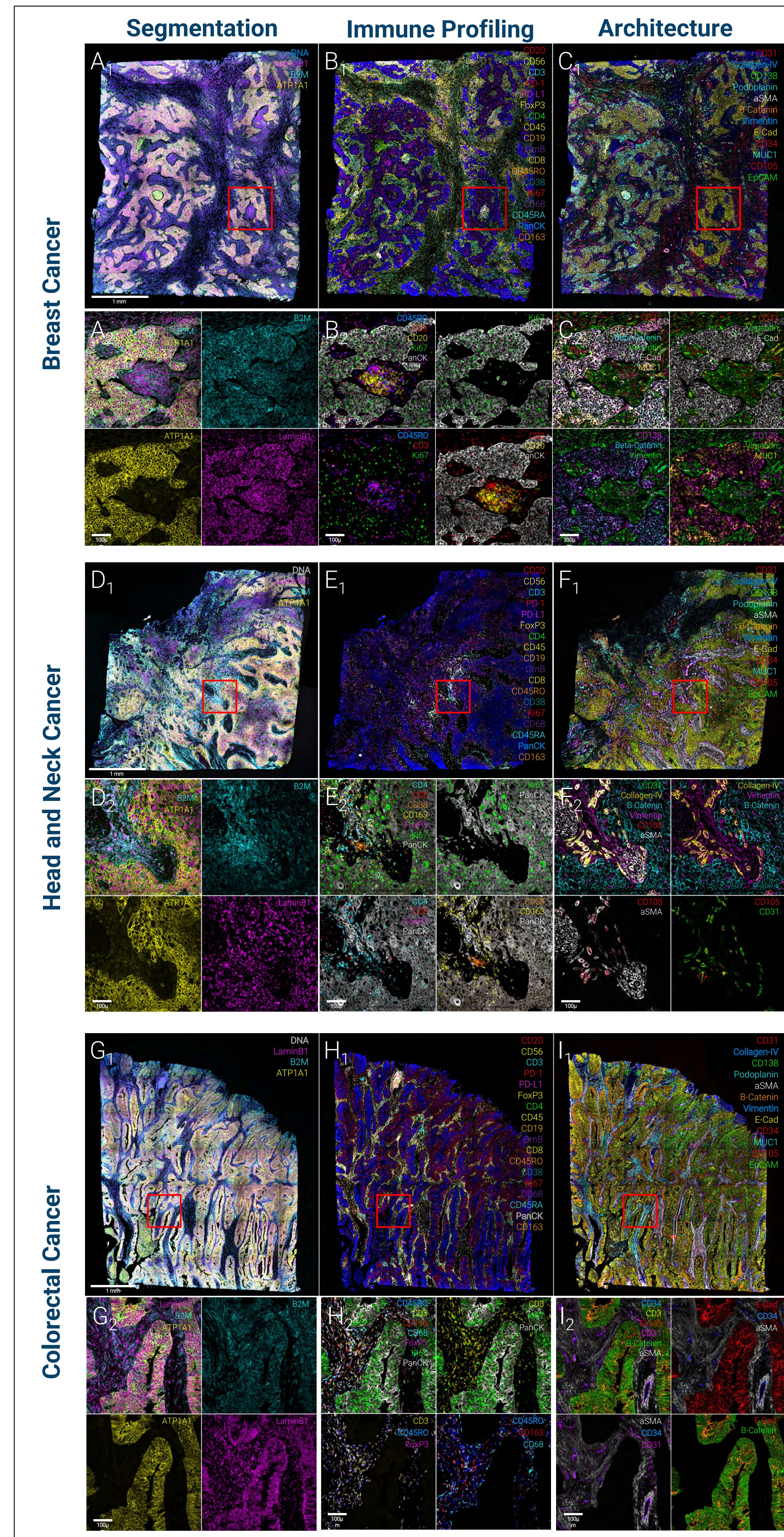


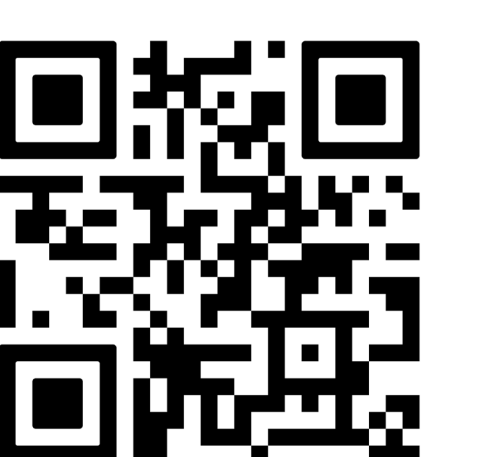
Figure 5. End-to-end analysis: The Next-Generation VistaPlex panels achieved high quality segmentation, phenotyping, and spatial analyses. Precise segmentation (A-B) enabled accurate phenotyping by unsupervised clustering (C, D), which allowed for the downstream characterization of spatial neighborhoods (E) containing multiple cell types of interest in the context of the surrounding microenvironment (F, G).

Conclusions

- Next-generation antibody panels are compatible with the new EpicIF workflow.
- Modular combination of VistaPlex panels provided distinct information on relevant cell and tissue types, enabling broad coverage for deep phenotyping without *de novo* validation.
- Precise segmentation allowed robust phenotyping using an unsupervised method, dramatically increasing analysis throughput and opening the door for personalized medicine using spatial biology.

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