Design and Optimization of Mouse Markers for Use in Melanoma, Cardiac Applications #2493

FORTIS LIFE SCIENCES

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Introduction

As one of the fastest growing cancers globally, melanoma research generates a significant amount of interest. Metastatic melanoma, specifically, drives the need for more diverse therapeutic approaches as surgery alone is often not enough. Targeted immunotherapies are increasing as a result to combat these difficult to treat cancers and monoclonal antibody production is subsequently becoming more important. Spatial biology of human tissues has escalated in recent years, leading to many novel discoveries. However, available tools for multiplexing have evolved at a slower pace, driving the need for diverse biomarkers. As human tissue is precious and often difficult to obtain, the mouse model has become the dominate disease model. Unfortunately, quality human anti-mouse antibodies are still lacking in many research areas. Tissue-specific mIF panels utilizing mouse biomarkers were designed and evaluated (Table 1), demonstrating the interchangeability and specificity of these antibodies.

Methods

Human anti-mouse targets of importance in melanoma and cardiac tissues were identified and separate tissue-specific panels were designed. Multiplex immunofluorescent manual staining was achieved using Bethyl Laboratories IHC-validated primary antibodies, Bethyl HRP-conjugated secondary antibodies, and Akoya Opal™ Polaris 7-color IHC kit fluorophores (Akoya Biosciences [NEL861001KT]). The melanoma panel consisted of F4/80, CD8a, CD31, SOX10, and CD4. Conversely, the cardiac panel included ASMA, CD31, F4/80, MEF2A, and ATP5A1.

Antibody	Reactivity	Catalog #	Panel Significance
Sox10	Hu, Ms	A700-080	transcription factor; melanoma marker
CD4	Ms	A700-167	helper T-cells
CD8a	Ms	A700-173	cytotoxic T-cells
CD3e	Ms	A700-175	total T-cell count
CD31	Ms	A700-180	endothelial marker
F4/80	Ms	A700-209	macrophage marker
ASMA	Hu, Ms	A700-082	cardiac fibroblast; myofibroblast marker
MEF2A	Hu, Ms	A303-531	cardiomyocyte marker
ATP5A1	Hu, Ms	A304-939	mitochondrial marker

Table 1. Selection of mouse-validated antibodies used in the various validation studies. Red indicates a polyclonal antibody. CD31 was utilized in both panels.

Optimization of antibody concentration and staining order was achieved using FFPE mouse immune tissue microarray serial sections. Each target was stained in either the first, third, or sixth position of the multiplexed panel order and subjected to heat-induced epitope retrieval (HIER). The final optimized order was then tested on mouse melanoma primary tumors and cardiac cross-sections in seven-color mIF. Whole slide scans were generated using the PhenoImager HT™ and subsequently analyzed via InForm™ and R® Studio.

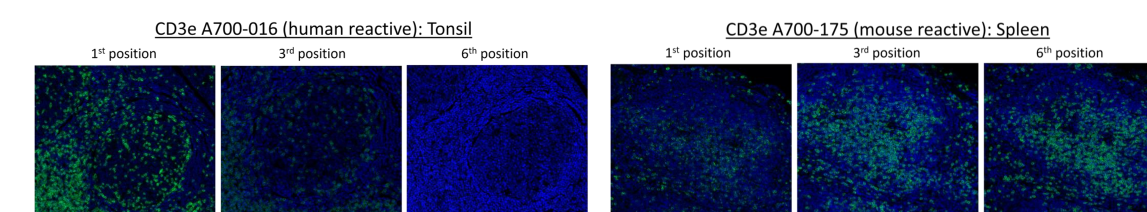

Figure 1. Significant difference in signal optimization between human-reactive CD3e and mouse-reactive CD3e antibodies observed during the order-testing protocol of assay optimization.

Process

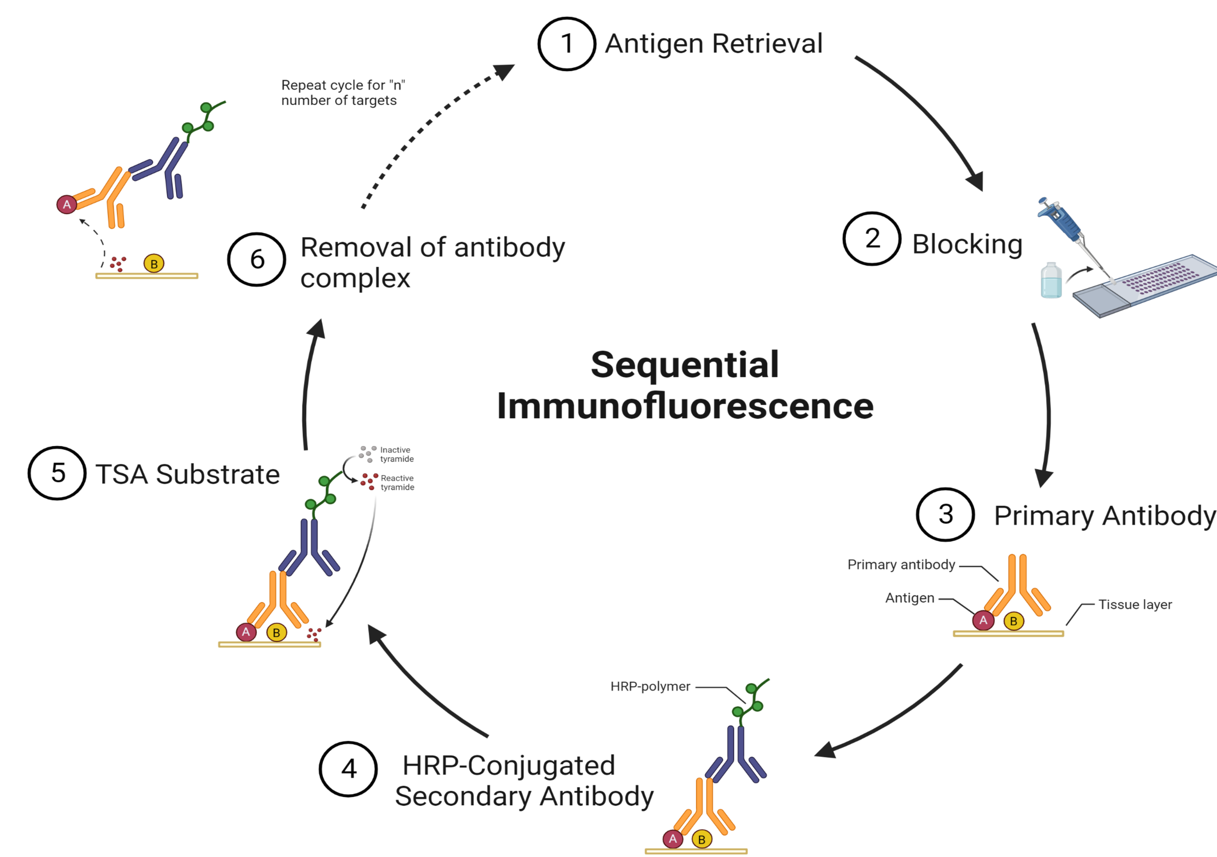

Figure 2. The sequential immunofluorescence method of manual staining using Akoya Opal™ reagents on each panel.

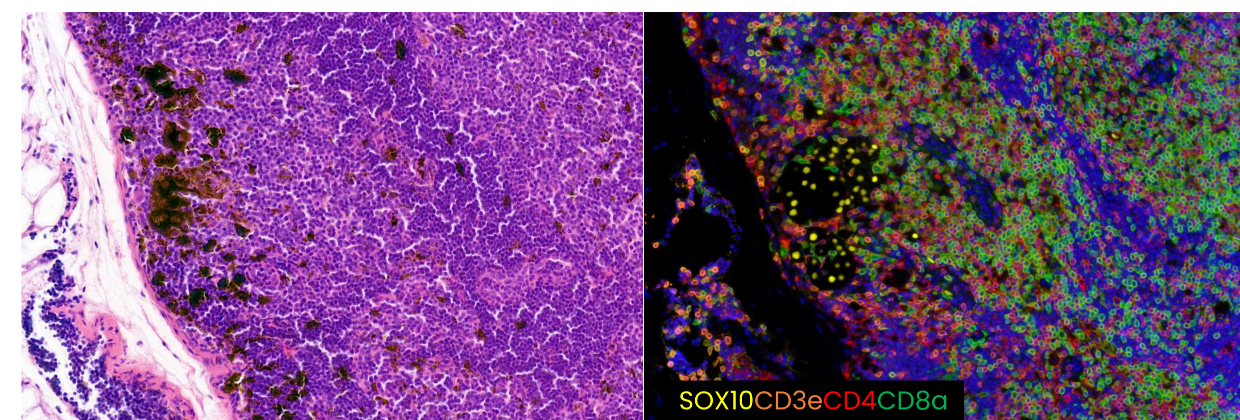

Figure 3. H&E stains are performed prior to multiplex immunofluorescence runs to enable side-by-side visuals of melanocytes and the associated SOX10 signal.

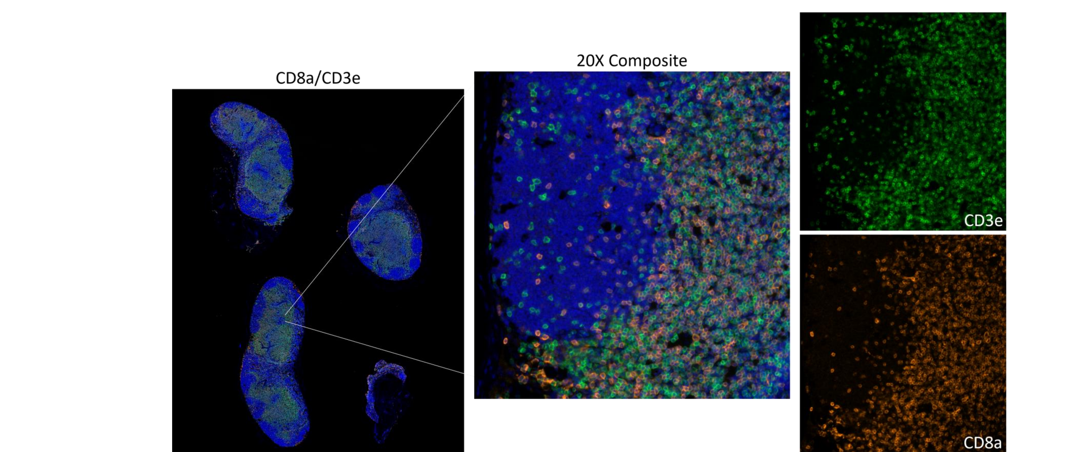

Figure 4. Separation of individual channels is done to assess autofluorescence, spectral overlap, and appropriate localization for each target.

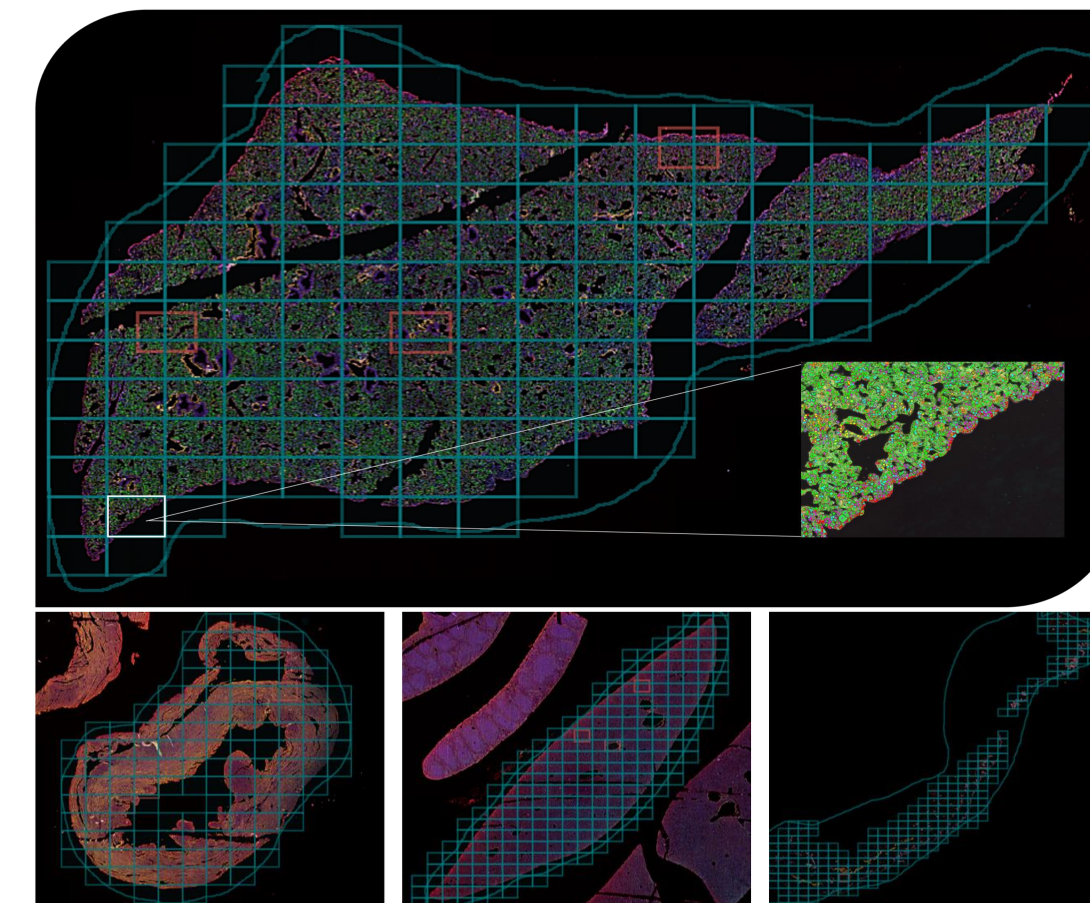

Figure 5. Tissue sections are stamped for AI training (red) and then ROIs are generated (blue) by drawing around the tissue of interest for batch analysis. Each ROI is analyzed and each cell assigned a phenotype in InForm™ (inset).

Assay Transferability

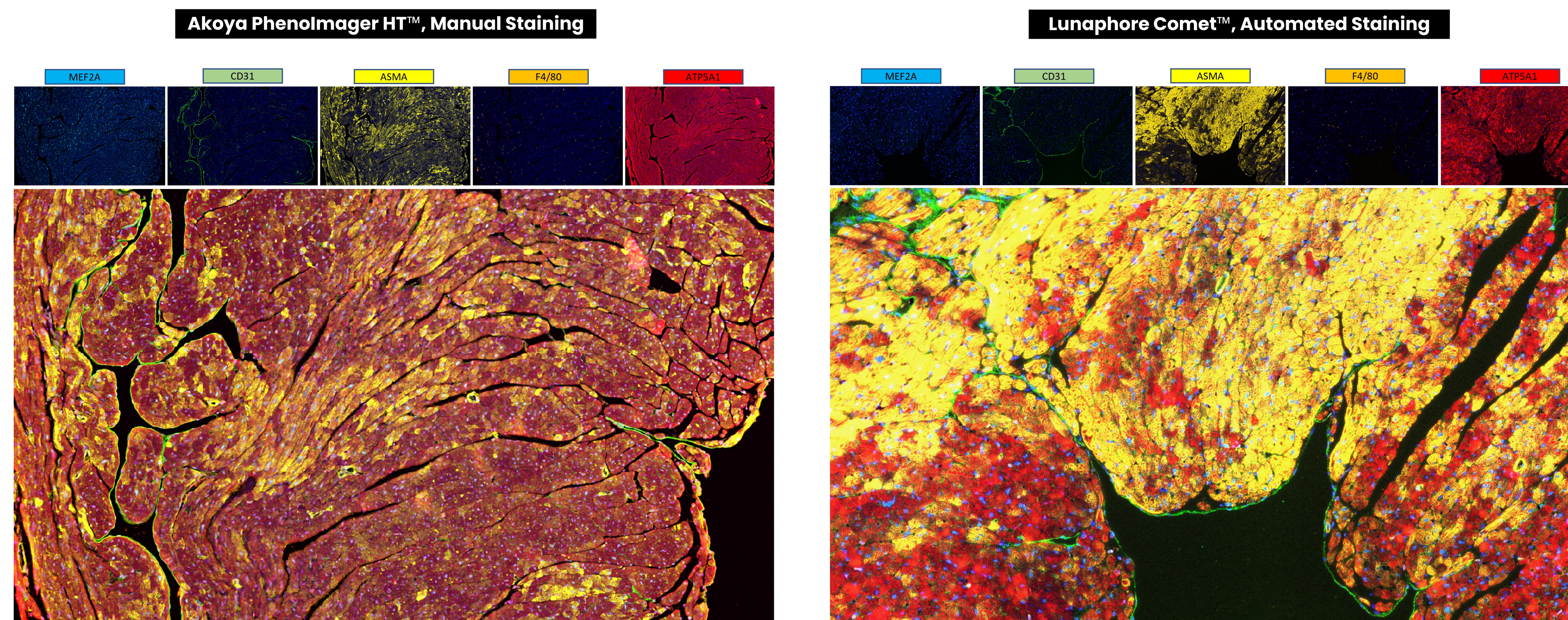

Figure 6. Comparison of standardized heart panel across two different staining modes and imaging systems. The detection of mouse ASMA (yellow), CD31 (green), F4/80 (orange), MEF2A (aqua), and ATP5A1 (red) is observed on mouse heart tissues using the PhenoImager HT (left) and Comet (right). The markers are accurately represented (appropriately localized) in both systems, though different tissues can display varying levels of each target and standardization on one system does not always effectively translate to another. Optimization of primary antibody concentrations and antigen retrieval methods play important roles when translating antibody panels across systems.

Results

High expression of SOX10 in melanoma is indicative of a poor prognosis clinically, making this marker significantly important in a diagnostic setting. Total SOX10 cell counts were elevated (40%) in mouse melanoma tissue, as compared to normal mouse skin tissue (Figure 7).

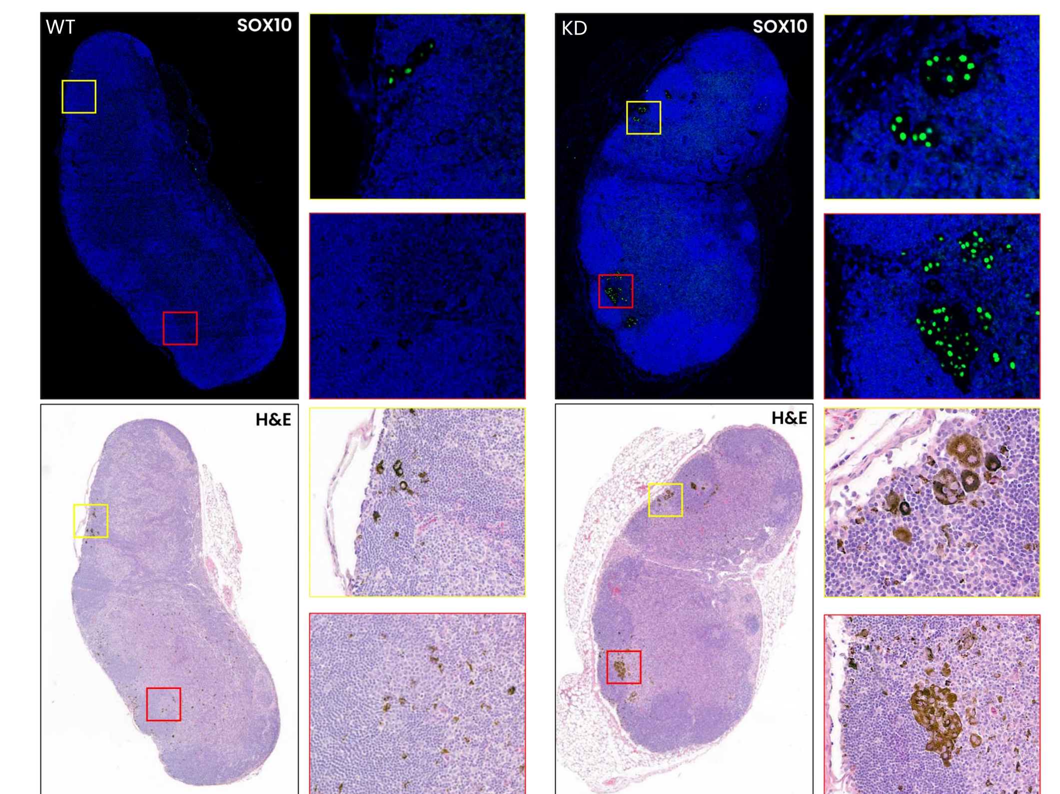

Figure 7. Expression of SOX10 in wild type vs. knockdown mouse lymph node tissues.

Antibodies identified for the cardiac panel were cross-evaluated against various mouse tissues (liver, skin, lung). Results were consistent with expected levels of each protein in their respective tissues: CD31 is prevalent in liver while levels are lower in epithelial tissue (skin).

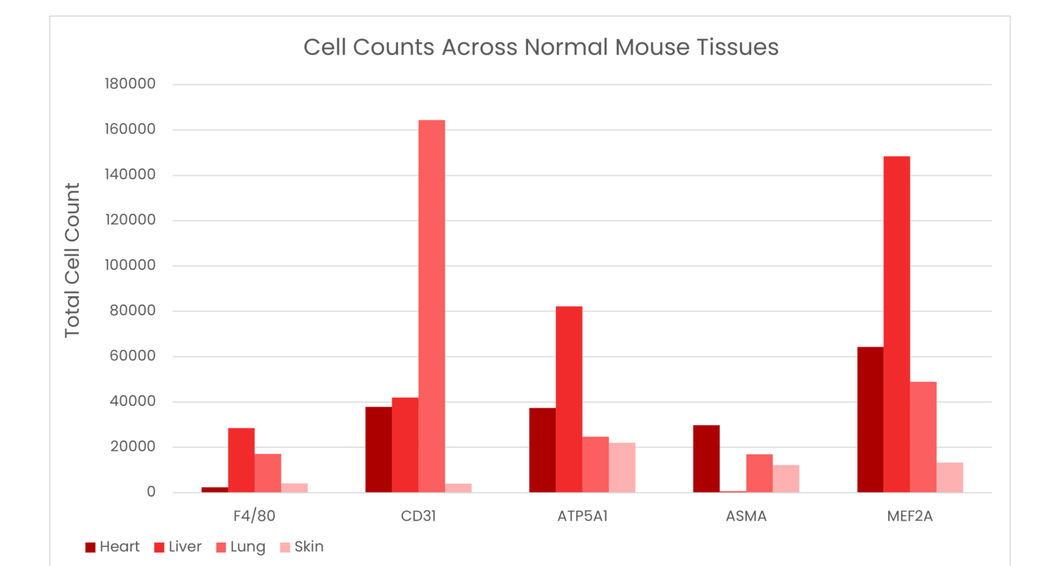

Figure 8. Total cell counts of mouse cardiac markers F4/80, CD31, ATP5A1, ASMA, and MEF2A across normal mouse tissues. The panel of markers identified for use in mouse cardiac studies can be used in desired mouse tissues with confidence in correct localization; similar validation studies were used for the melanoma panel.

Conclusions

The identification of unique biomarkers involved in cancer development and metastasis is of increasing importance. When examined in conjunction with the tumor microenvironment in a spatial biology context, these novel targets can provide insight into general tumor composition, prognoses, and treatment options. Here we examined the ability of rabbit antibodies to detect mouse protein in multiplex IHC experiments. Rabbit antibodies, which are either designed for mouse targets or cross-react with mouse targets, are effective reagents to develop mouse multiplex panels. This also aids in closing the gap between human tissue spatial biology experiments and those conducted in mouse models.

Acknowledgements

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