

# Detection of radiopharmaceuticals and their cold surrogates by Imaging Mass Cytometry enables assessment of single-cell functional response, therapeutic biodistribution, and modulation of the immune microenvironment

Jennifer L. Gorman<sup>1</sup>, Felix B. Salazar<sup>2</sup>, Kevin Wyszatko<sup>3</sup>, Michael J. Geuenich<sup>1</sup>, Smriti Kala<sup>4</sup>, Thom G.A. Reuvers<sup>5</sup>, Matthew Watson<sup>1</sup>, Daniel Majonis<sup>4</sup>, Hang Zhou<sup>4</sup>, Bao Ying Chen<sup>2</sup>, Christopher Heskett<sup>2</sup>, Marjolijn Hameetman<sup>6</sup>, Qanber Raza<sup>4</sup>, Sheila Singh<sup>7</sup>, Christina Loh<sup>4</sup>, James Mansfield<sup>4</sup>, Julie Nonnekens<sup>5</sup>, Erik de Blois<sup>8</sup>, Kieran R. Campbell<sup>1</sup>, Saman Sadeghi<sup>3</sup>, Anna M. Wu<sup>2</sup>, and Hartland W. Jackson<sup>1</sup>

<sup>1</sup>Lunenfeld-Tanenbaum Research Institute, Toronto, Canada; <sup>2</sup>Department of Immunology & Theranostics, Beckman Research Institute of City of Hope, Duarte, USA; <sup>3</sup>Department of Chemistry & Chemical Biology, McMaster University, Hamilton, Canada; <sup>4</sup>Standard BioTools Canada Inc., Markham, Canada; <sup>5</sup>Department of Molecular Genetics and Radiology & Nuclear Medicine, Erasmus Medical Center Cancer Institute, Rotterdam, Netherlands; <sup>6</sup>Flow Cytometry Core Facility, Leiden University Medical Center, Leiden, Netherlands; <sup>7</sup>Centre for Discovery in Cancer Research, McMaster University, Hamilton, ON; <sup>8</sup>Radiology & Nuclear Medicine, Erasmus Medical Center, Rotterdam, Netherlands.

Abstract: 7196

## Background and Objectives

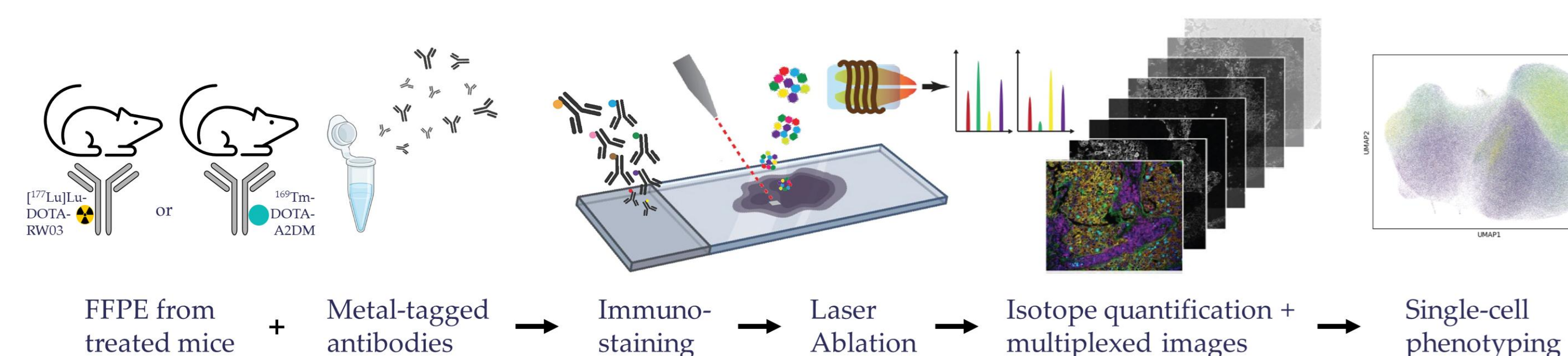
- Conventional imaging techniques like PET and SPECT measure localized isotope uptake; however, they lack the resolution to assess single-cell biodistribution and can't assess cellular measures of treatment response<sup>1,2</sup>
- Imaging Mass Cytometry (IMC) technology combines laser tissue ablation with mass spectrometry readouts and can measure metal-based chemotherapies in tissue<sup>3-6</sup>

### Objectives:

- Benchmark IMC detection of metal from RPTs and cold surrogates
- Measure whole tumor and single-cell spatial distribution of metal-conjugated therapeutics using IMC technology
- Quantify impact of therapeutic dose and IMC staining workflow on amount of retained metal
- Assess potential utility of multiplexed single-cell measurement of therapeutic response

## Methods

- Autoradiography and IMC signal detection were compared by spotting serial dilutions on an agarose-coated slide and assessing radioactivity and metal intensity in the same spot
- Two therapeutics were assessed: RW03 Ab targeting human anti-CD133<sup>7</sup> radiolabeled with <sup>177</sup>Lu and anti-PSCA (prostate stem cell antigen) A2DM antibody fragment<sup>8</sup> conjugated to <sup>169</sup>Tm (cold surrogate)
- Mice bearing HT29 xenografts were treated with [<sup>177</sup>Lu]Lu-DOTA-RW03 (3.8 or 18.5 MBq) and hPSCA knock-in mice bearing KPC-hPSCA xenografts were treated with <sup>169</sup>Tm-A2DM (10, 20, or 100 µg)
- Both single-cell segmentation and pixel-based analysis were carried out to assess therapeutic distribution and cellular response
- IMC panels included tissue context markers (stromal cells, epithelial cells, vessels, ECM), along with markers of therapeutic response (DNA damage, apoptosis, proliferation)



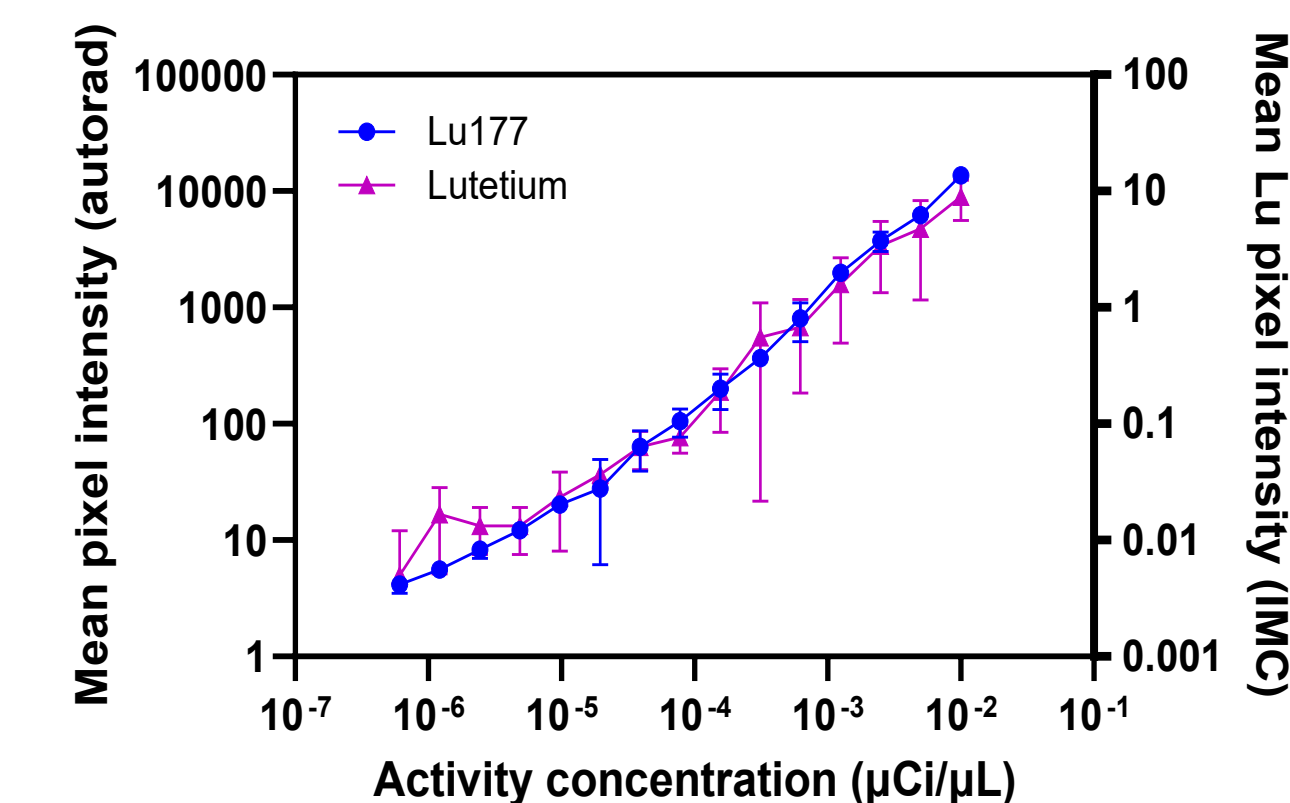
**Figure 1:** Schematic of IMC workflow.<sup>9-11</sup> Tumor tissue was harvested from mice injected with an RPT targeting CD133 or a cold surrogate targeting PSCA. Following IMC staining (+/- Ab) and acquisition, machine learning tools were used to segment cells and identify complex single-cell phenotypes and spatial distribution

## Results

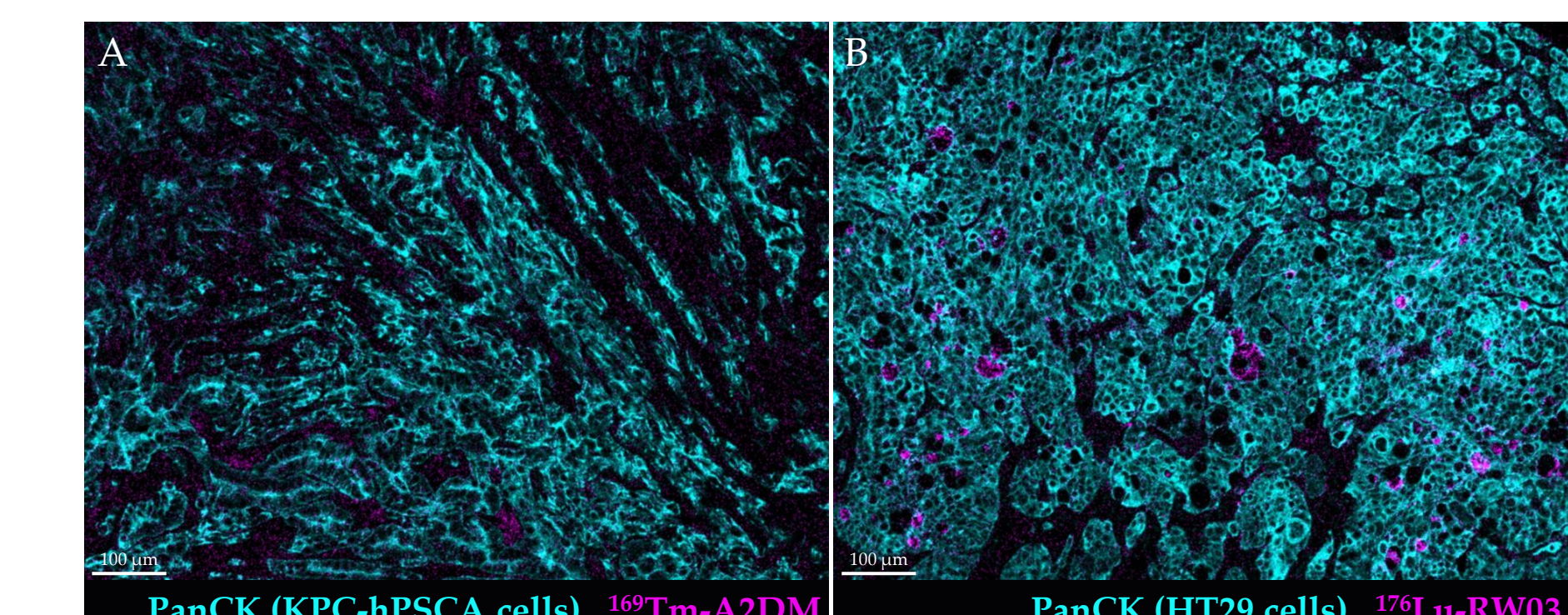
### Benchmarking IMC detection

Lutetium (175 + 176) detection using IMC technology was highly correlated with autorad detection of lutetium-177 from the same spot ( $r = 0.9943$ ,  $p < 0.0001$ )

**Figure 2:** Correlation of autorad and IMC signal detection



### Detection of RPT or cold surrogate in tumor tissue using IMC

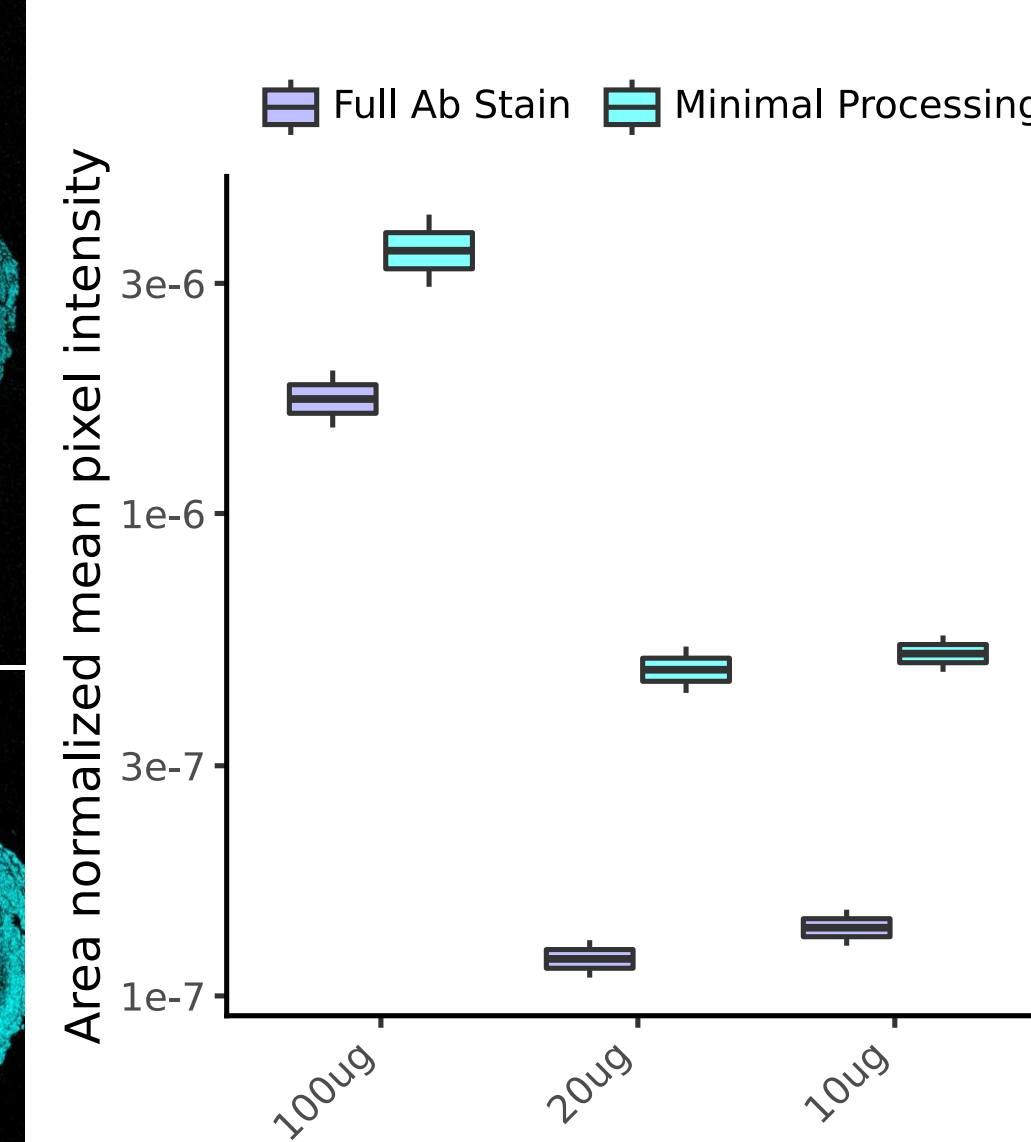
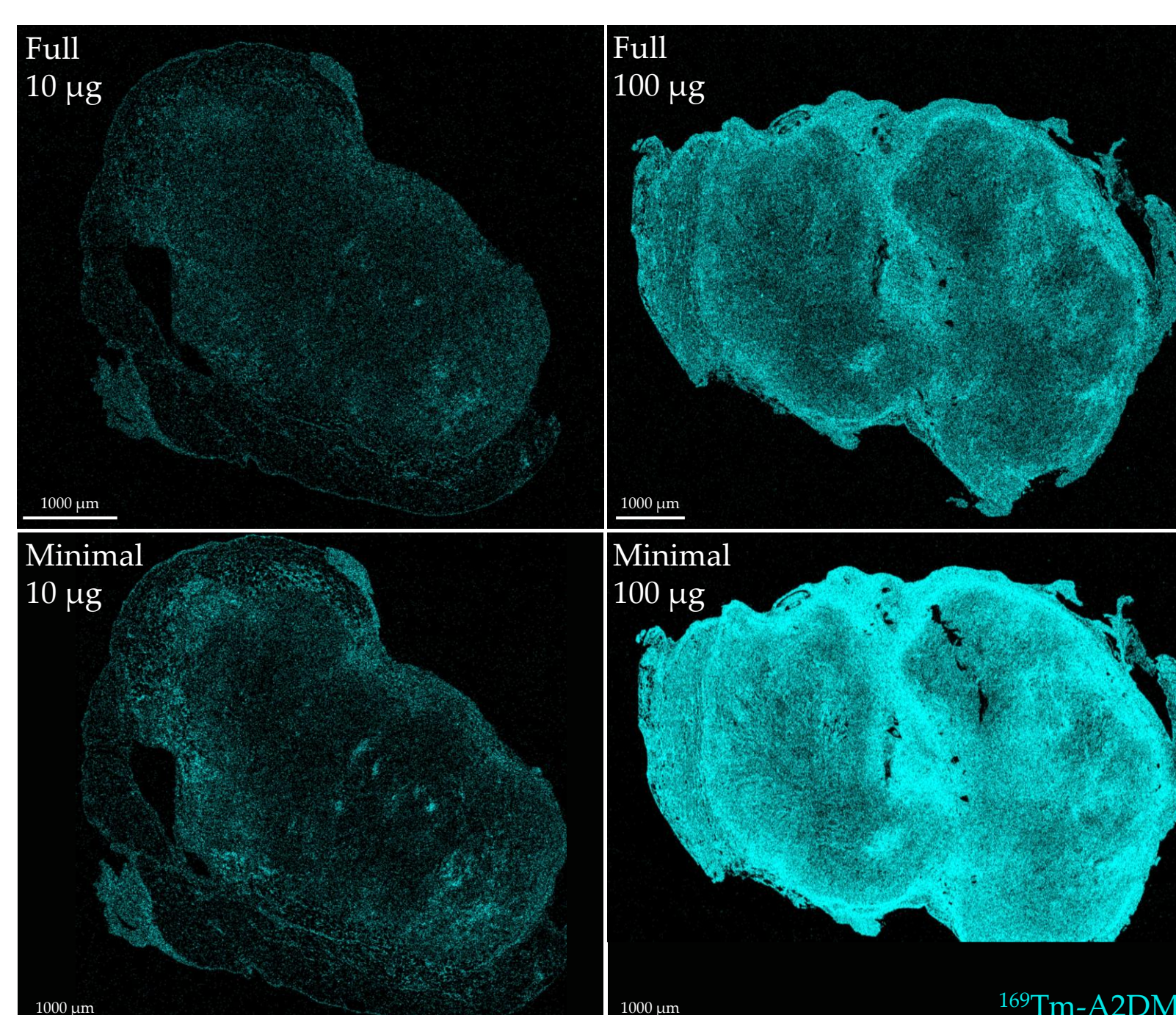


<sup>169</sup>Tm-A2DM cold surrogate (A) and <sup>176</sup>Lu from carrier-added <sup>177</sup>Lu-RW03 (B) were detected in xenograft tumors using IMC

**Figure 3:** IMC images showing PanCK tumor expression and retained metal signal from cold surrogate (A) and carrier-added RPT metal (B)

## Results

### Whole tumor biodistribution of <sup>169</sup>Tm-A2DM RPT cold surrogate

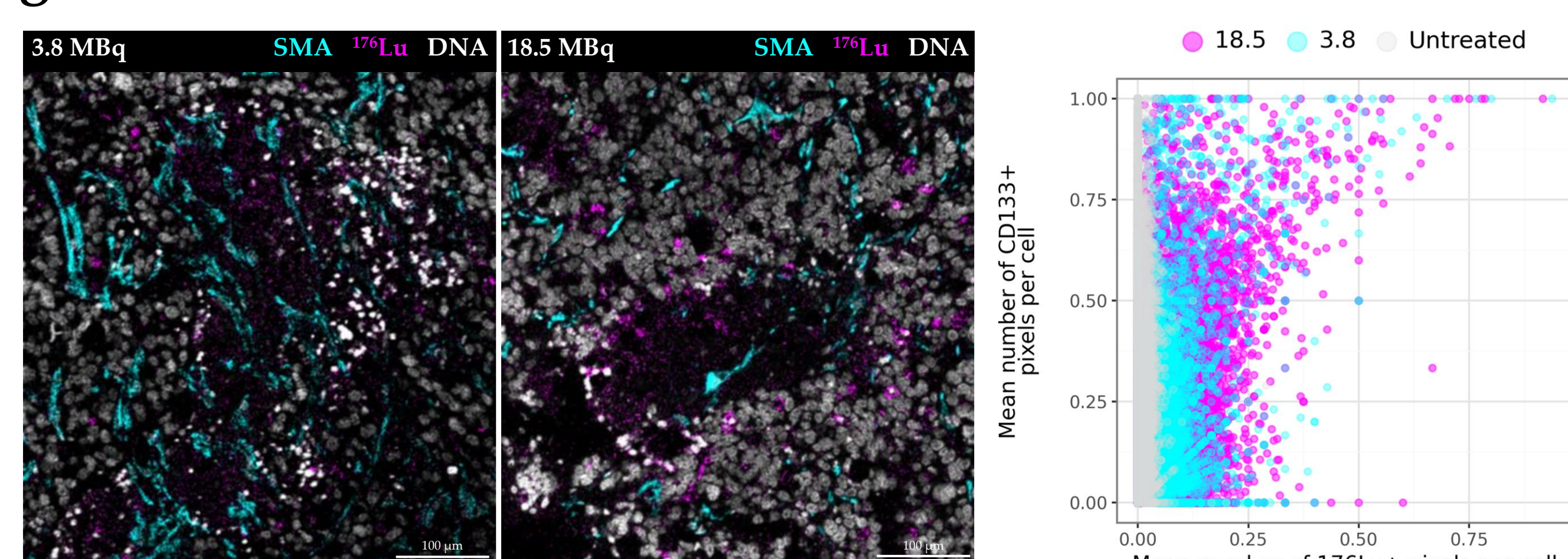


**Figure 4:** IMC images and pixel intensity quantification of cold surrogate for different therapeutic doses and IMC staining workflows

- Pixel intensity from <sup>169</sup>Tm-A2DM tumor-retained metal was increased with a higher therapeutic dose
- Full IMC Ab staining workflow decreased the amount of tumor-retained metal across all doses examined

### Single-cell biodistribution of <sup>177</sup>Lu-RW03 RPT

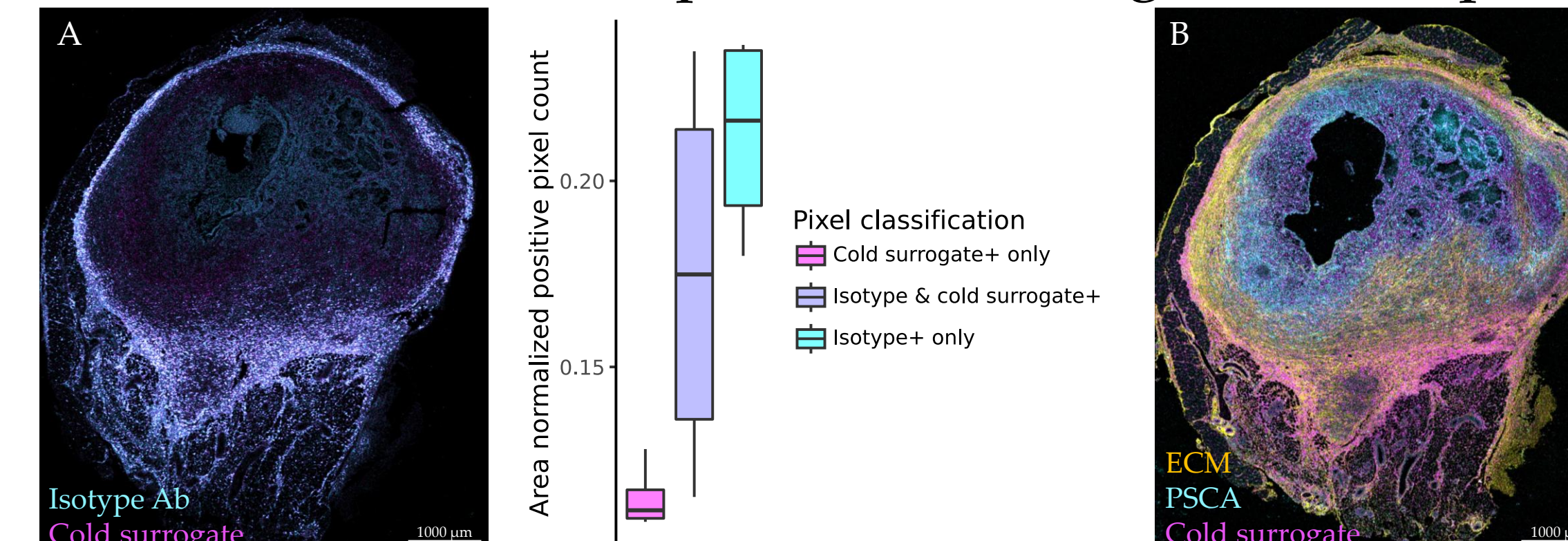
- Retained carrier metal from both doses of [<sup>177</sup>Lu]Lu-DOTA-RW03 were detectable by IMC
- An increase in <sup>176</sup>Lu+ pixels per cell was observed in mice treated with the higher dose



**Figure 5:** IMC images and quantification of <sup>176</sup>Lu+ pixels on CD133+ cells from tumors treated with 3.8 or 18.5 MBq of [<sup>177</sup>Lu]Lu-DOTA-RW03

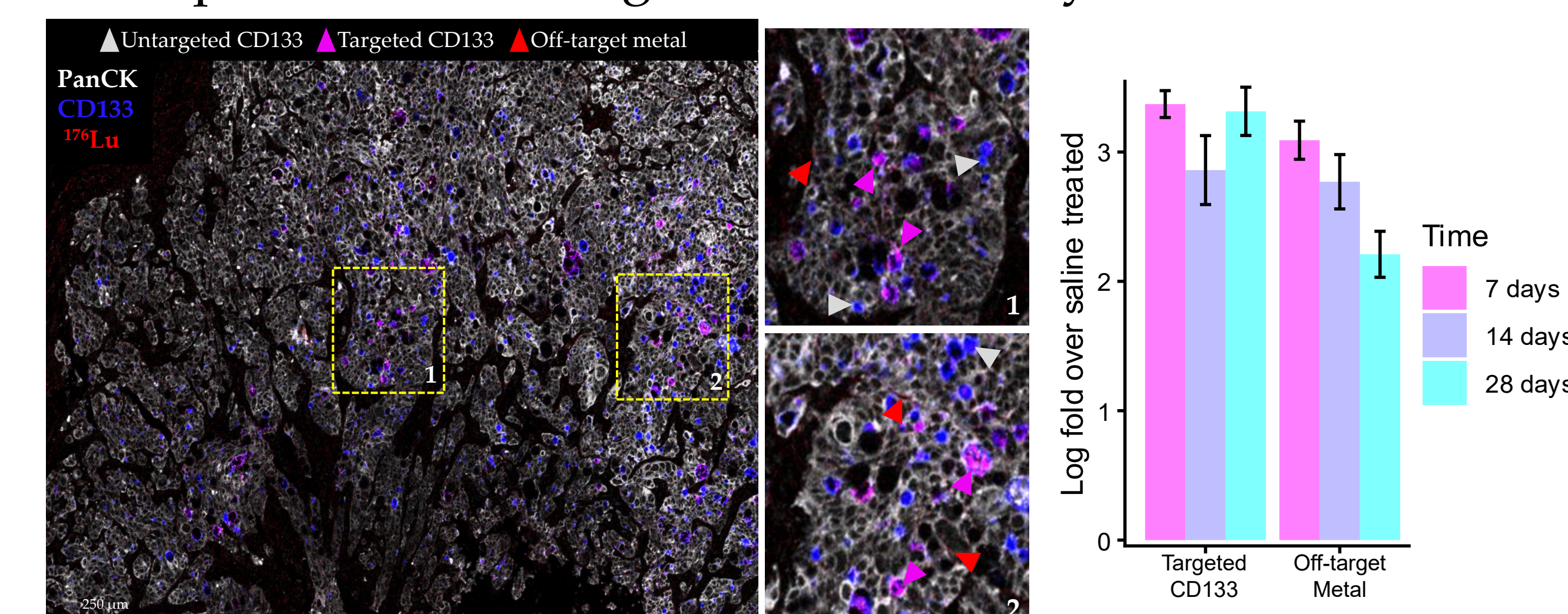
### Co-detection of therapeutic target and retained metal by IMC

<sup>169</sup>Tm-A2DM signal overlapped with an isotype control Ab in regions with low PSCA expression and high ECM deposition



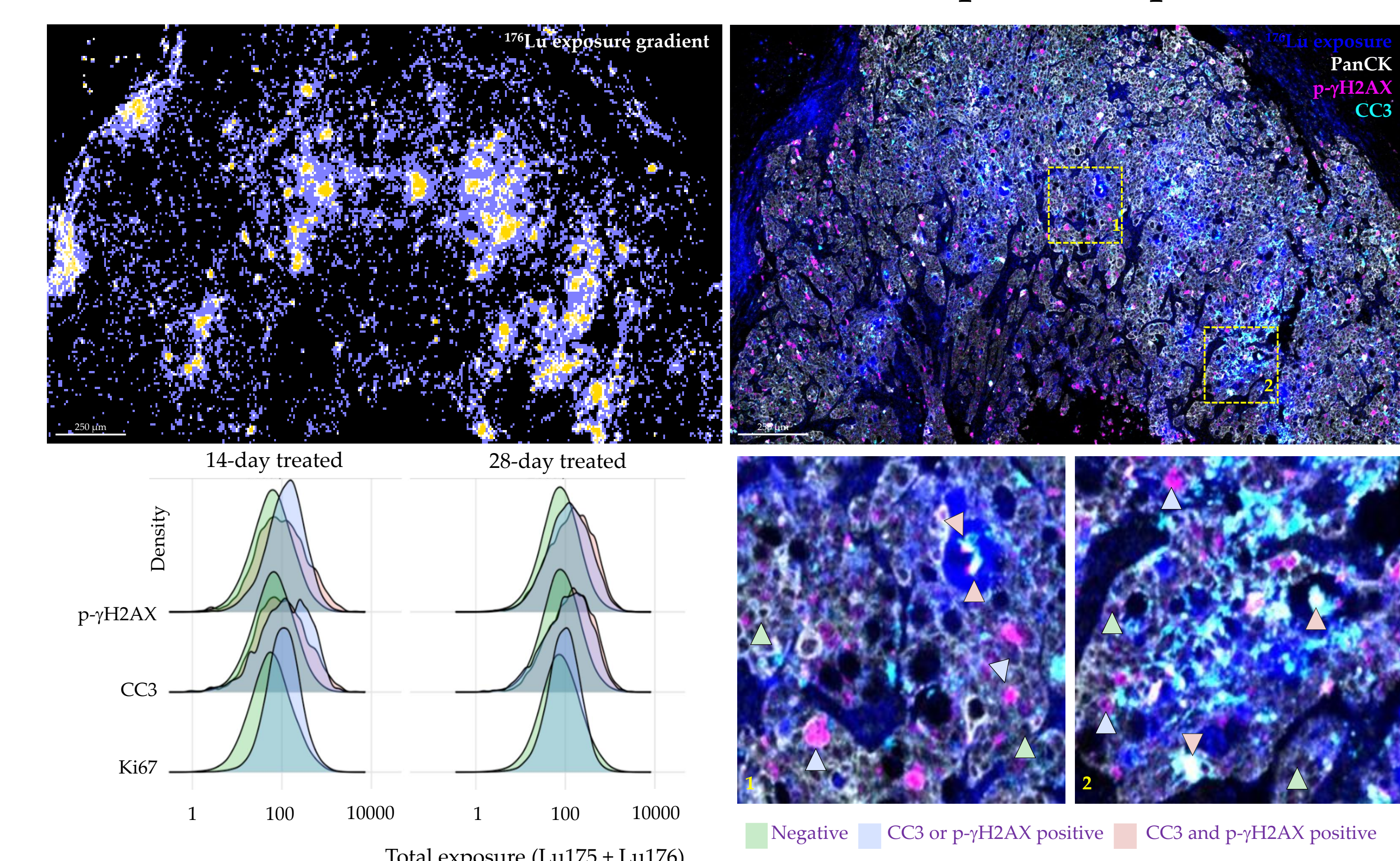
**Figure 6:** IMC images showing signal from <sup>169</sup>Tm-A2DM with (A) isotype control Ab and (B) PSCA and ECM markers. Plot shows quantification of isotype control and cold surrogate positive pixel overlap (n = 4 tumors).

Retained metal (<sup>176</sup>Lu) from <sup>177</sup>Lu-RW03 RPT showed distinct overlap with CD133 target across a 28-day time course



**Figure 7:** IMC images depicting co-localization of <sup>176</sup>Lu and CD133. Plot compares pixels positive for <sup>176</sup>Lu and CD133 (targeted CD133) vs <sup>176</sup>Lu alone (off-target metal)

### Measures of therapeutic response



**Figure 8:** IMC images visualizing lutetium exposure estimate and functional markers of response. Plot shows density of cells negative or positive for functional markers in relation to their total lutetium exposure at tissue harvest

- Lutetium pixel intensity was converted to a distance-based exposure estimate
- Post-treatment, cells positive for apoptosis (CC3), DNA damage (p-γH2AX), or both showed higher levels of lutetium exposure

## Summary

IMC technology enabled simultaneous detection of RPT metal or cold surrogate, target, and functional markers of response in the same tissue section, which could allow for in situ measurements of drug distribution to expand our current understanding of the microenvironmental response to RPTs.