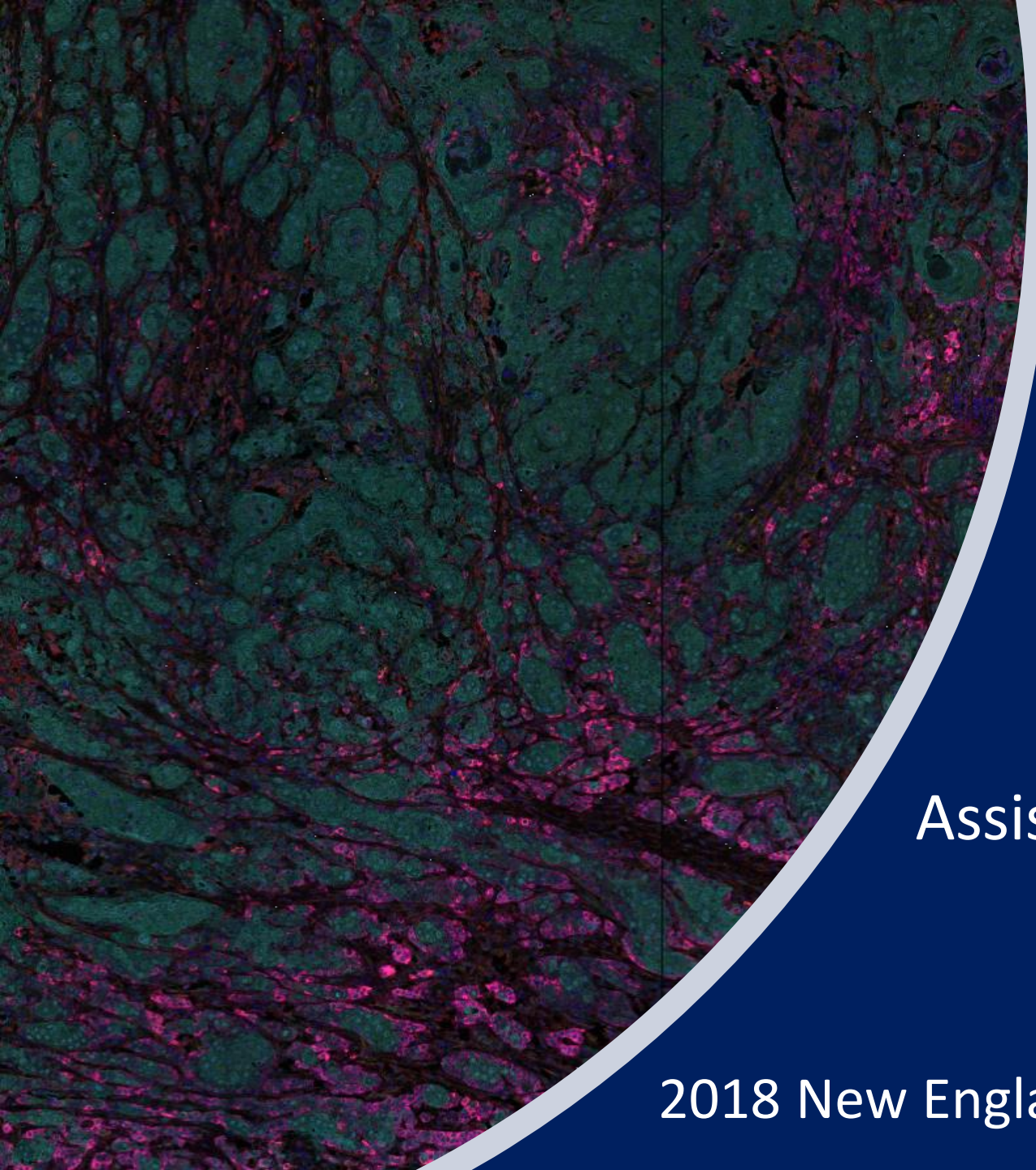

# Multiplexed Multispectral Imaging of Tumor Tissue

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Department of Medicine, HMS

2018 New England Cytometry Users Group Fall Meeting

# Conflicts of Interest

**Advisory Board Positions**:
Merck Innovation Group
Seventh Sense Biosystems

**Scientific Advisor:**
Mantra Bio

**No patents of filings related to this work**

In collaboration with Brad Bernstein

# Drivers of the Work

João Oliveira-Costa, DDS, PhD

Anuraag Parikh, MD

Sid Puram, MD, PhD

In collaboration with Brad Bernstein

# Head and neck squamous cell carcinoma

- Squamous cell carcinoma of mucosal lining of the upper aerodigestive tract
- US: 3% of all cancers in 2018. Estimated 51540 new cases and 10030 deaths in 2018
- Oral cavity: most common subsite
- Primary surgery is standard
- 5-year survival of 64.8%

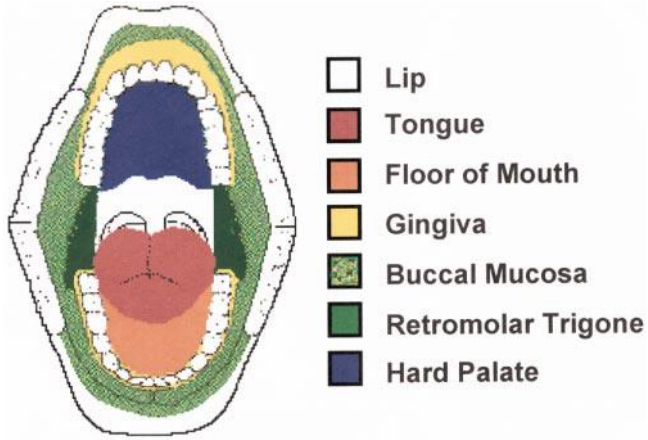
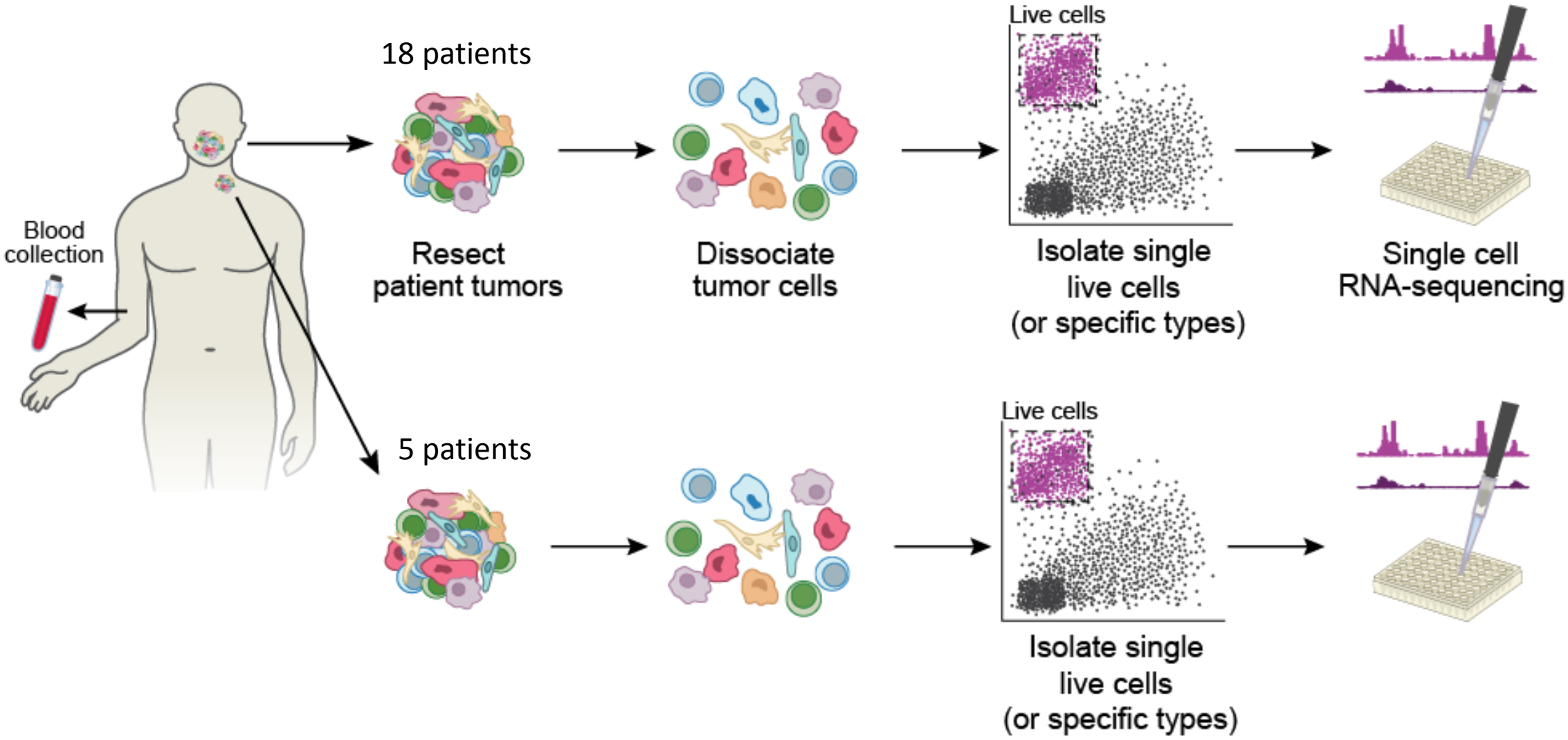



**Figure 5–1.** Diagram of the oral cavity and subsites.


*National Cancer Institute, [www.cancer.gov](http://www.cancer.gov)*

*Myers: Cancer of the Head and Neck (2003)*

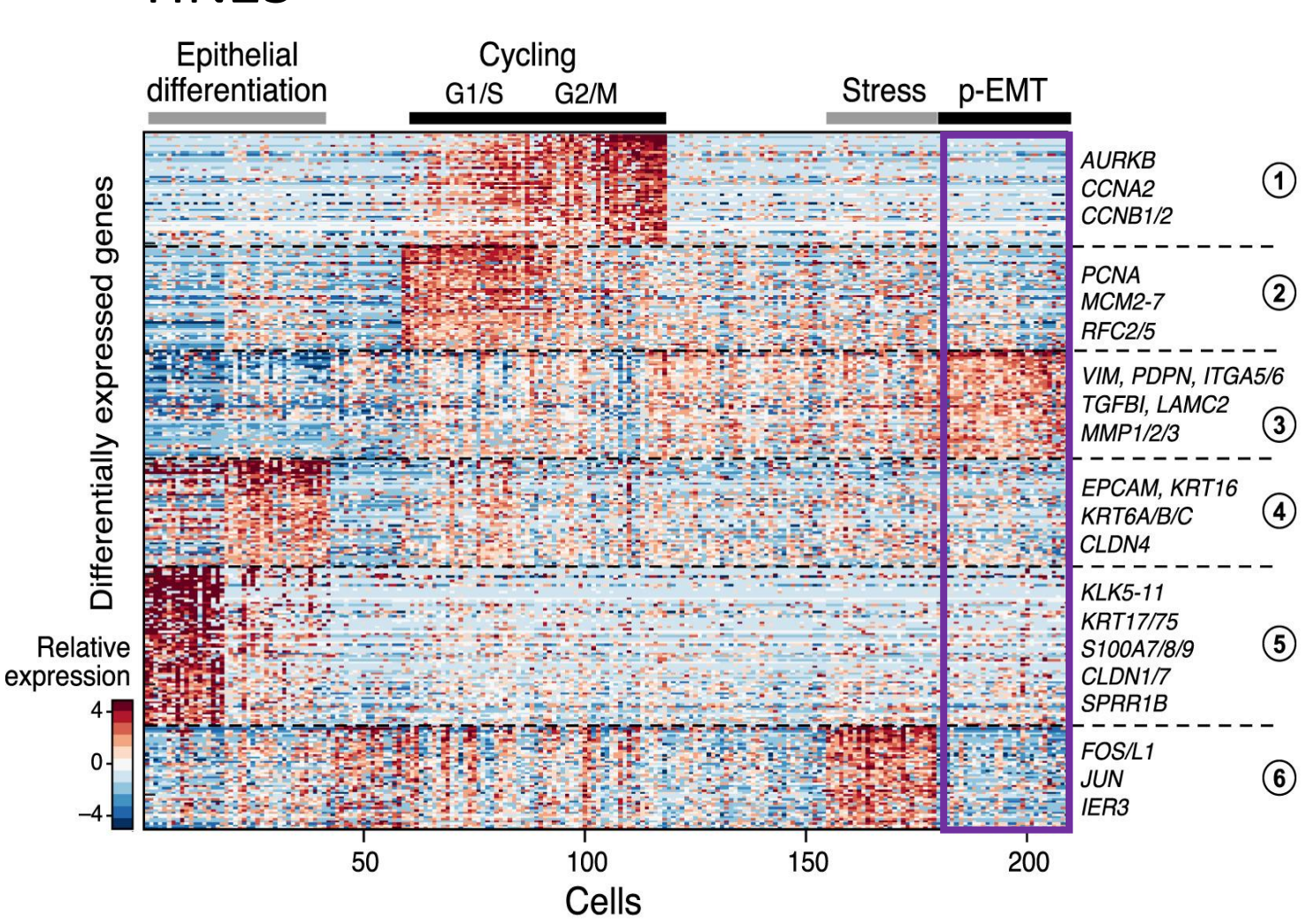
# Single cell RNA sequencing (scRNA-seq)

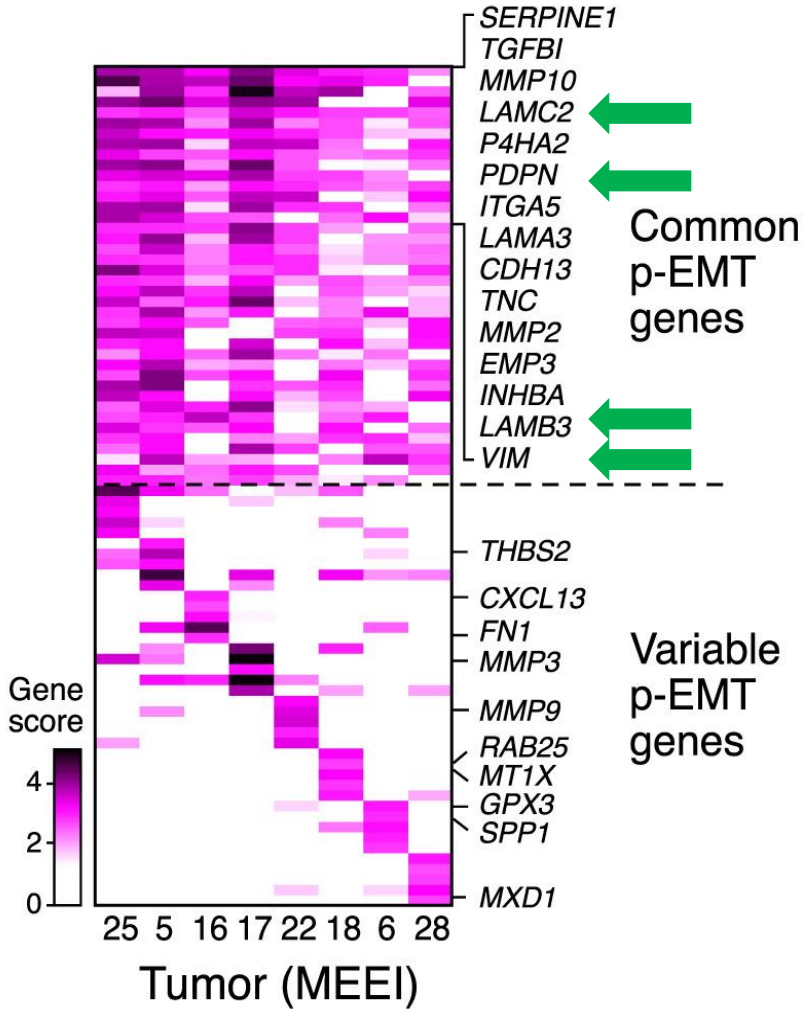



Puram*, Tirosh*, Parikh* et al, *Cell* (2017)

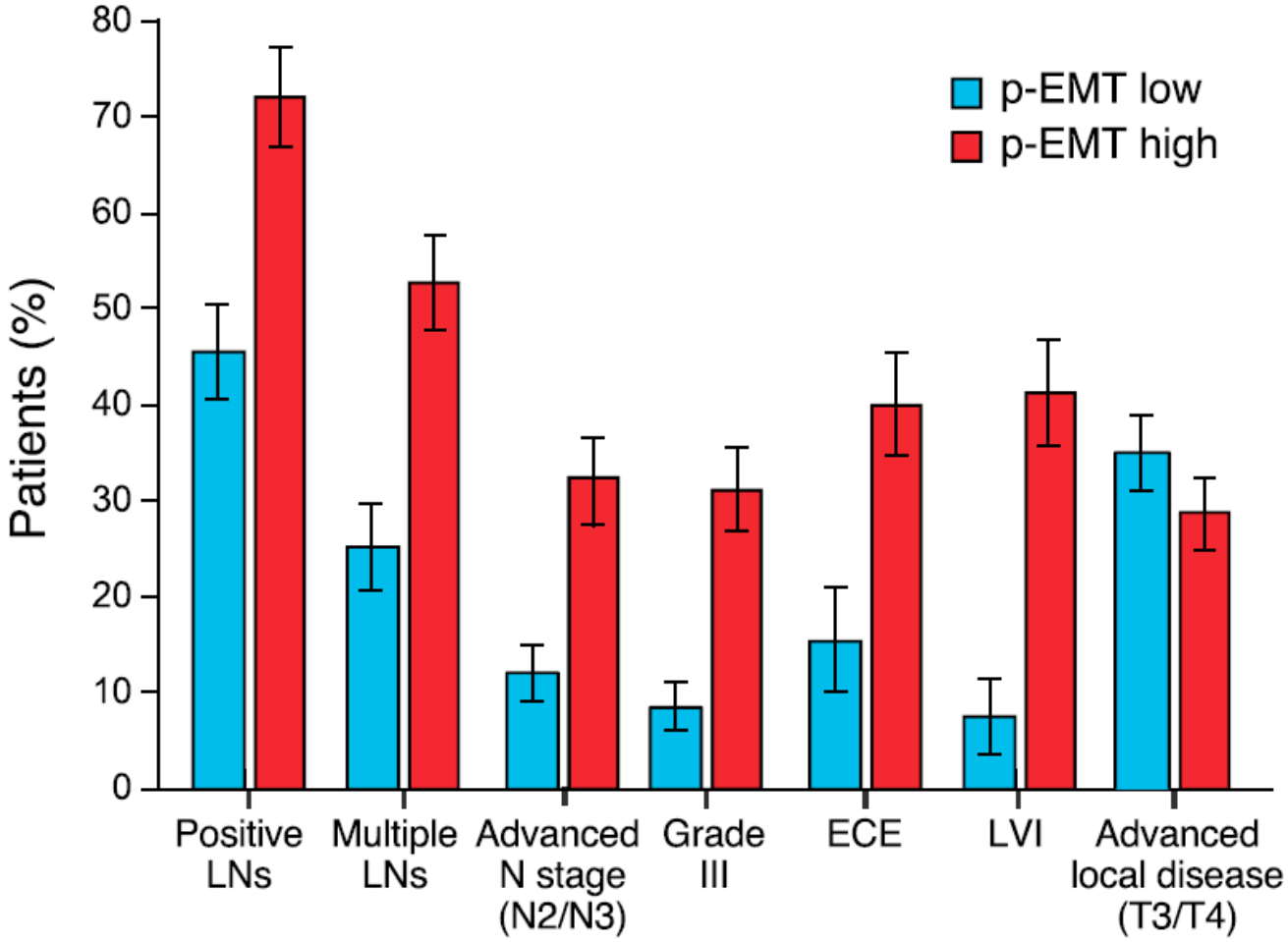
# Consistent programs across tumors

HN25



# p-EMT predicts nodal metastasis



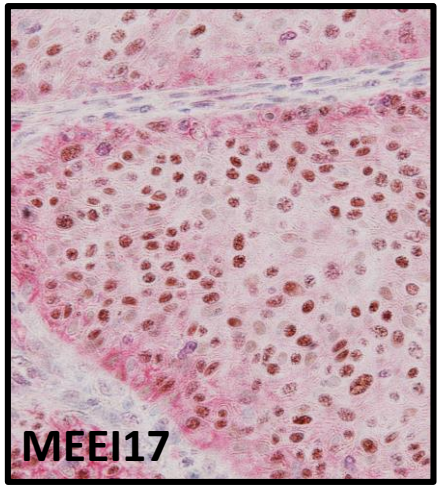

Puram*, Tirosh*, Parikh* et al, *Cell* (2017)

# Localization of p-EMT at the leading edge

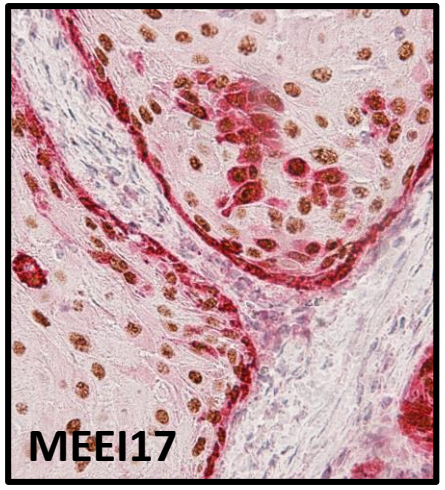
**p-EMT program at the invasive edge**

**Epi. Dif. at the core**

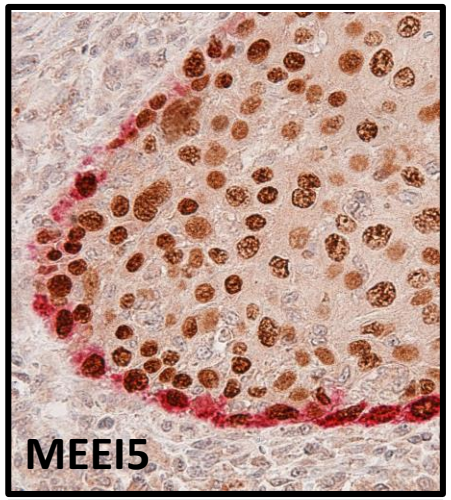
p63 / PDPN

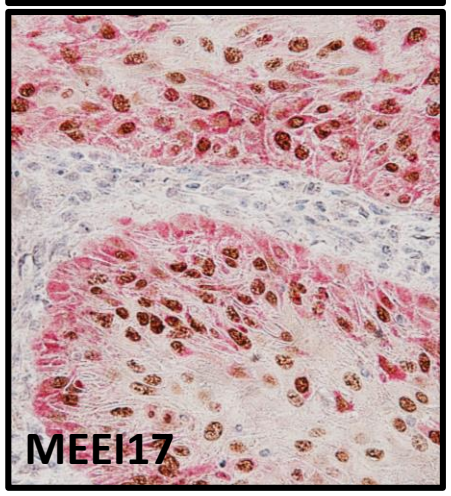




p63 / LAMB3

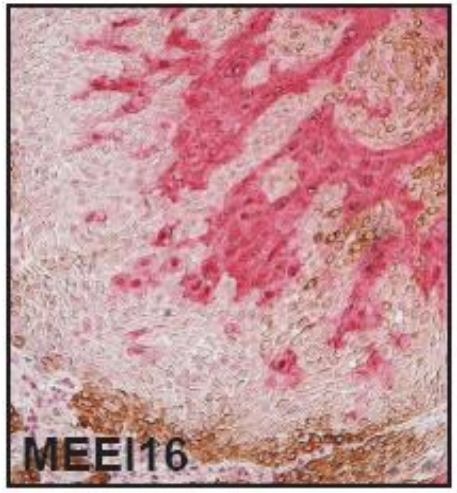



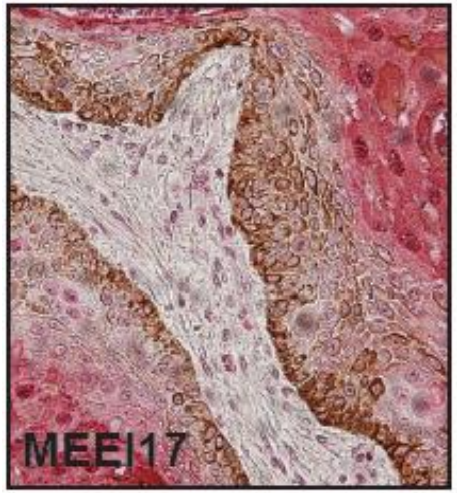

p63 / LAMC2




p63 / SPRR1B



# Summary

**p-EMT program** associated with metastasis:

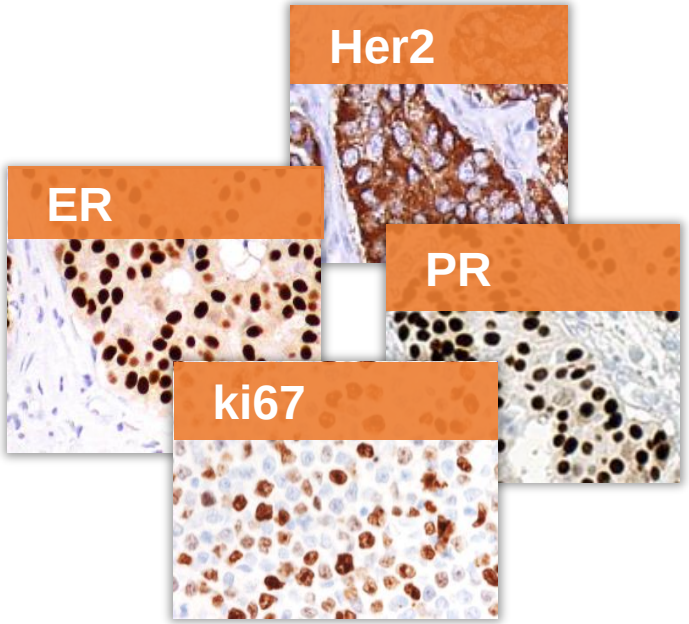
- Associated with invasiveness and metastasis
- Localized at leading edge in close apposition to CAFs

# MSI enables higher levels of analysis

## Simultaneous ER / PR / Her2 / ki67 in Breast Cancer Tissue

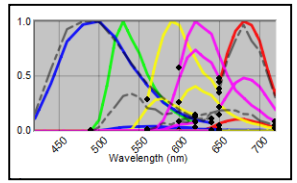
***Conventional single-plex***

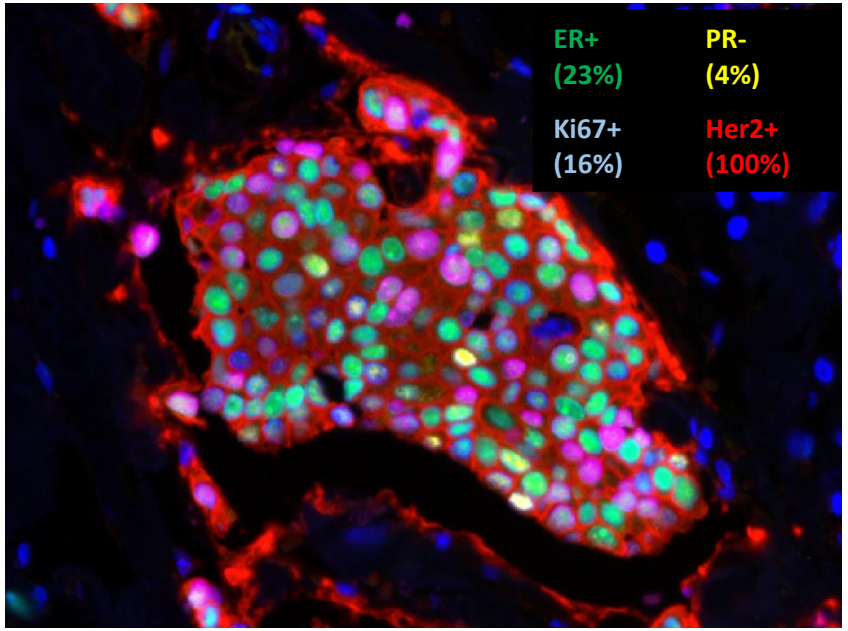
**visual, serial sections**




***Multi-plex assay***

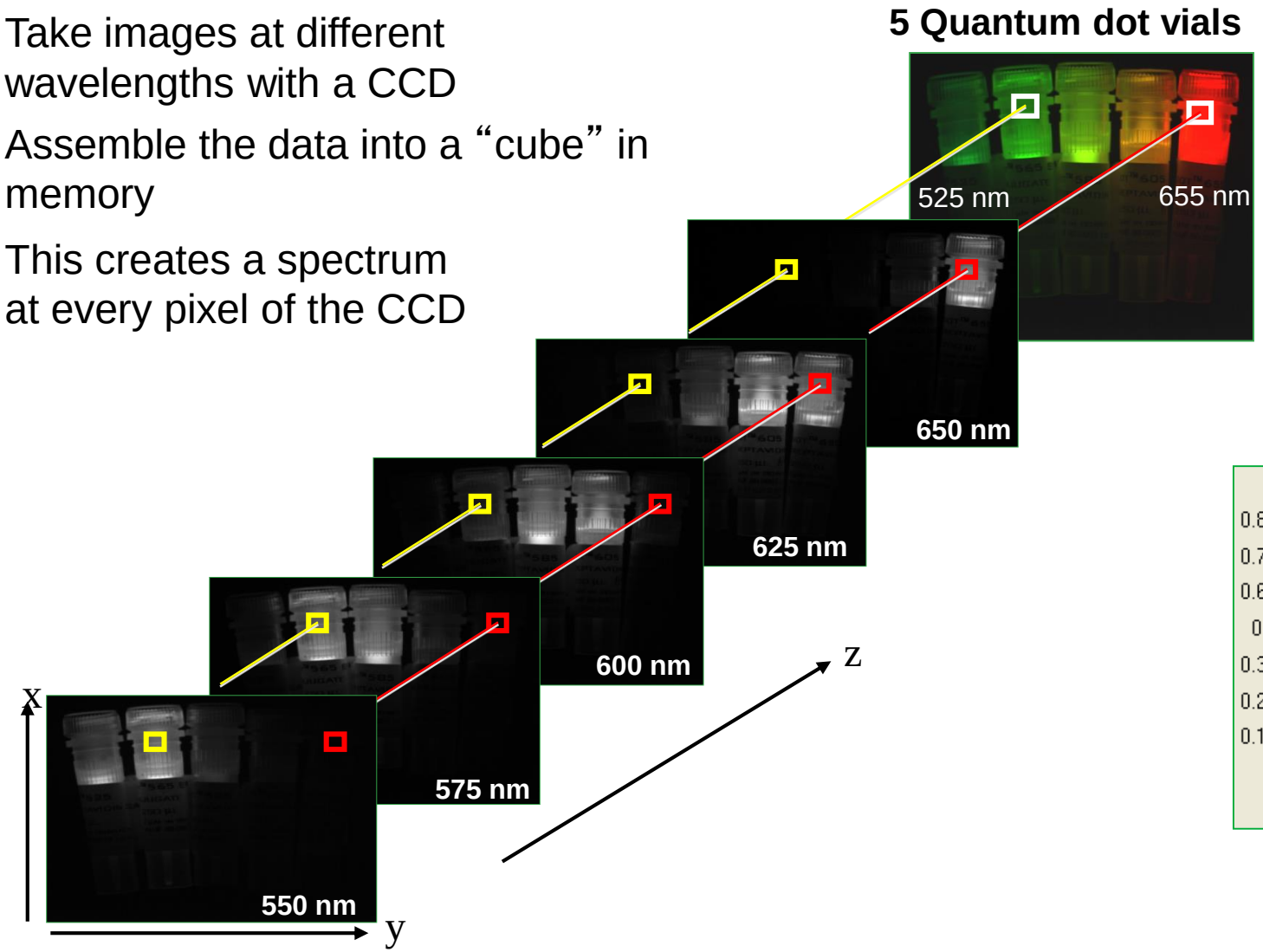
**automated, quantitative**

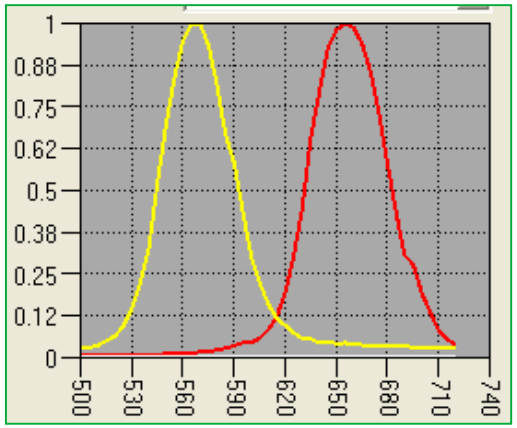


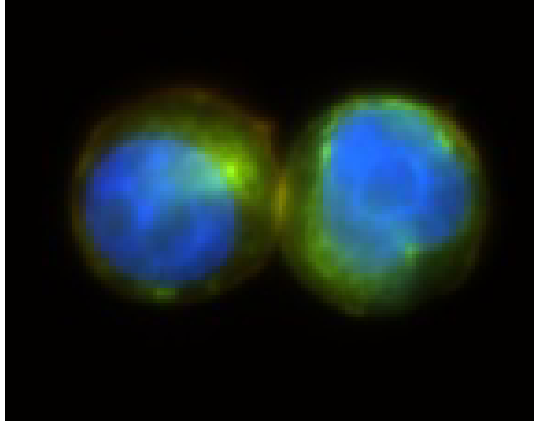



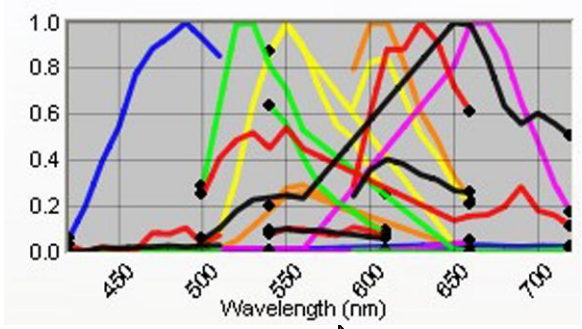
# Multispectral Imaging (MSI)

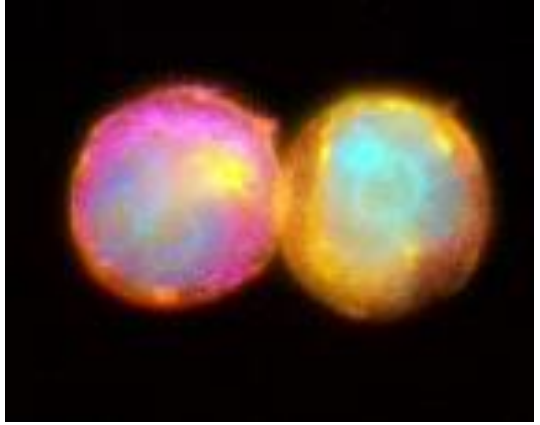
1. Take images at different wavelengths with a CCD
2. Assemble the data into a “cube” in memory
3. This creates a spectrum at every pixel of the CCD

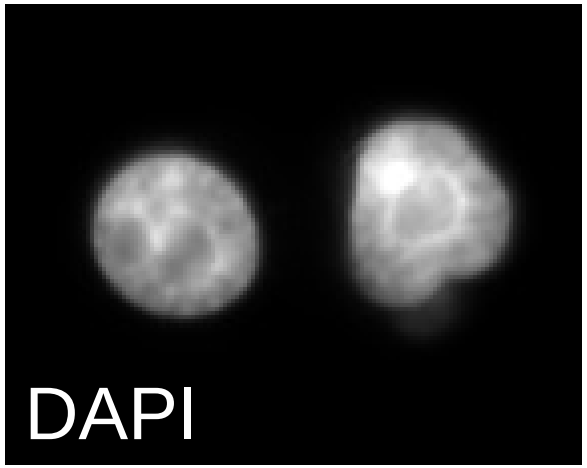


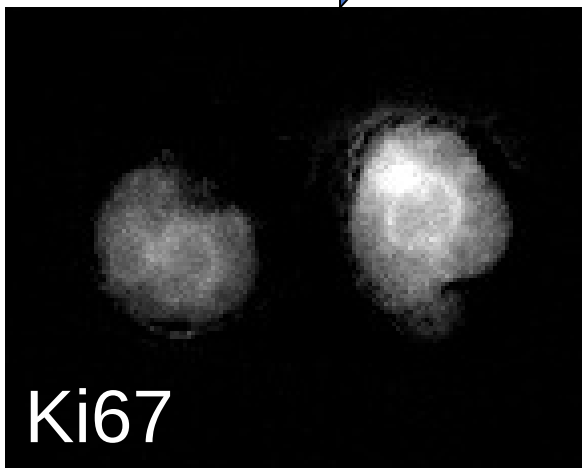



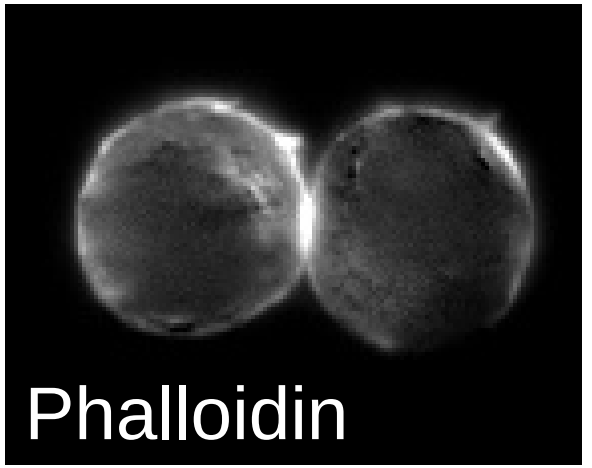


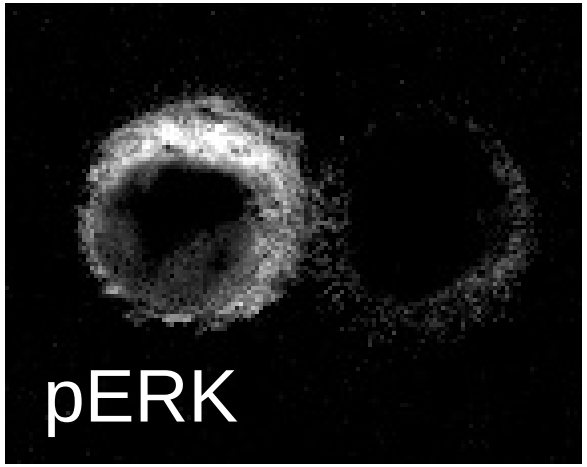

1.0
0.8
0.6
0.4
0.2
0.0
450
500
550
600
650
700
Wavelength (nm)




DAPI


Ki67


Phalloidin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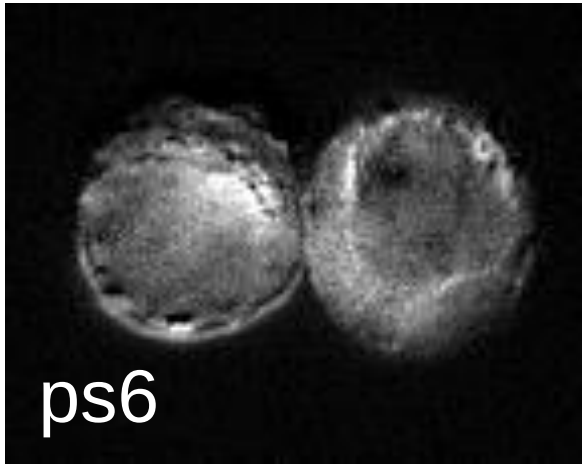
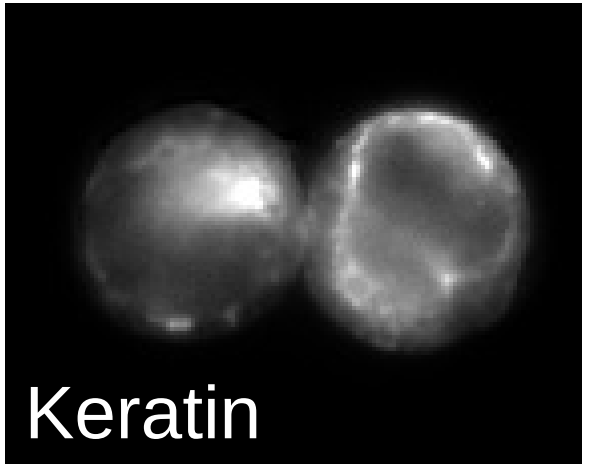
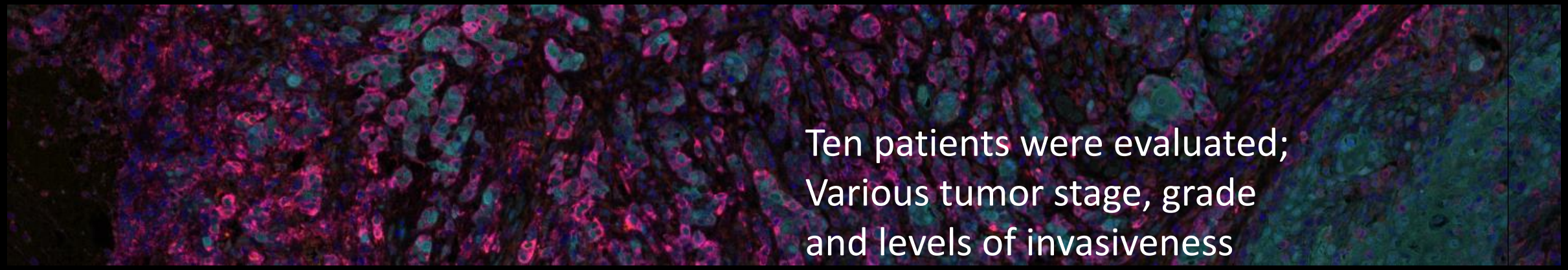
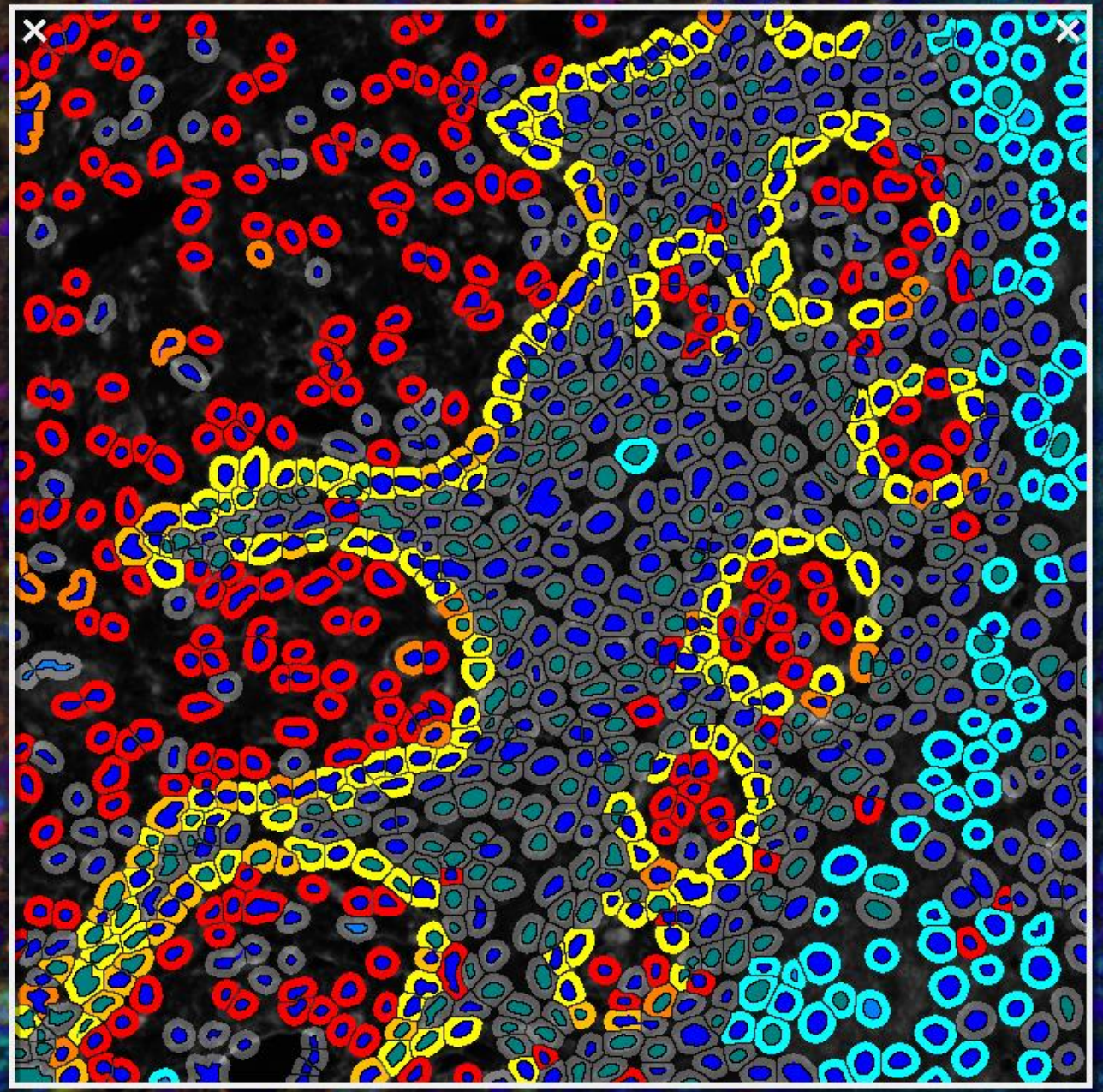

ps6


Keratin

# Head and Neck MSI Tissue Staining

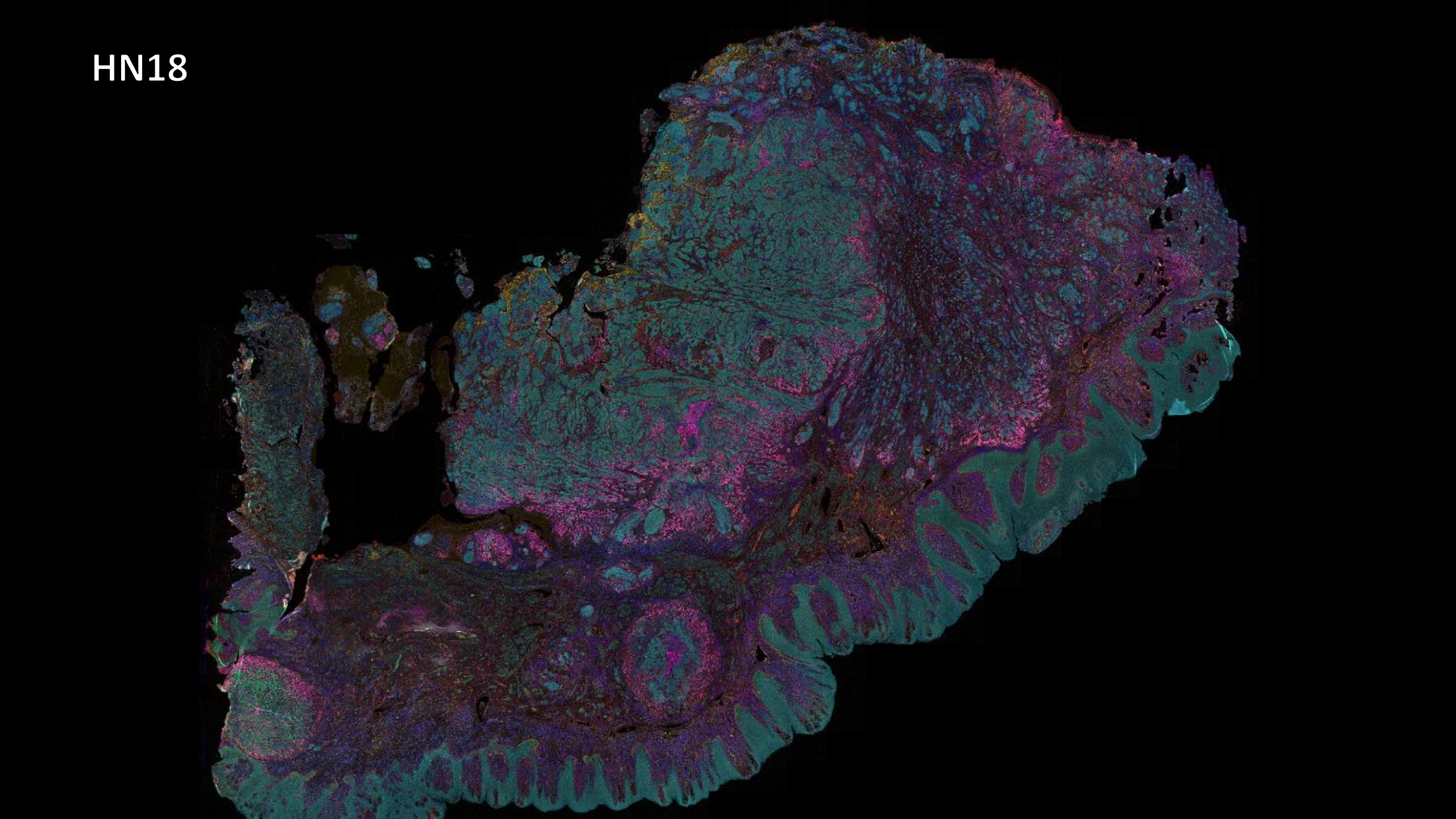
| Epithelial differentiation | p-EMT | Tumor |
|---|---|---|
| SPRR1B | LAMC2 | p63 |
| | LAMB3 | |
| | VIM | |
| | PDPN | |





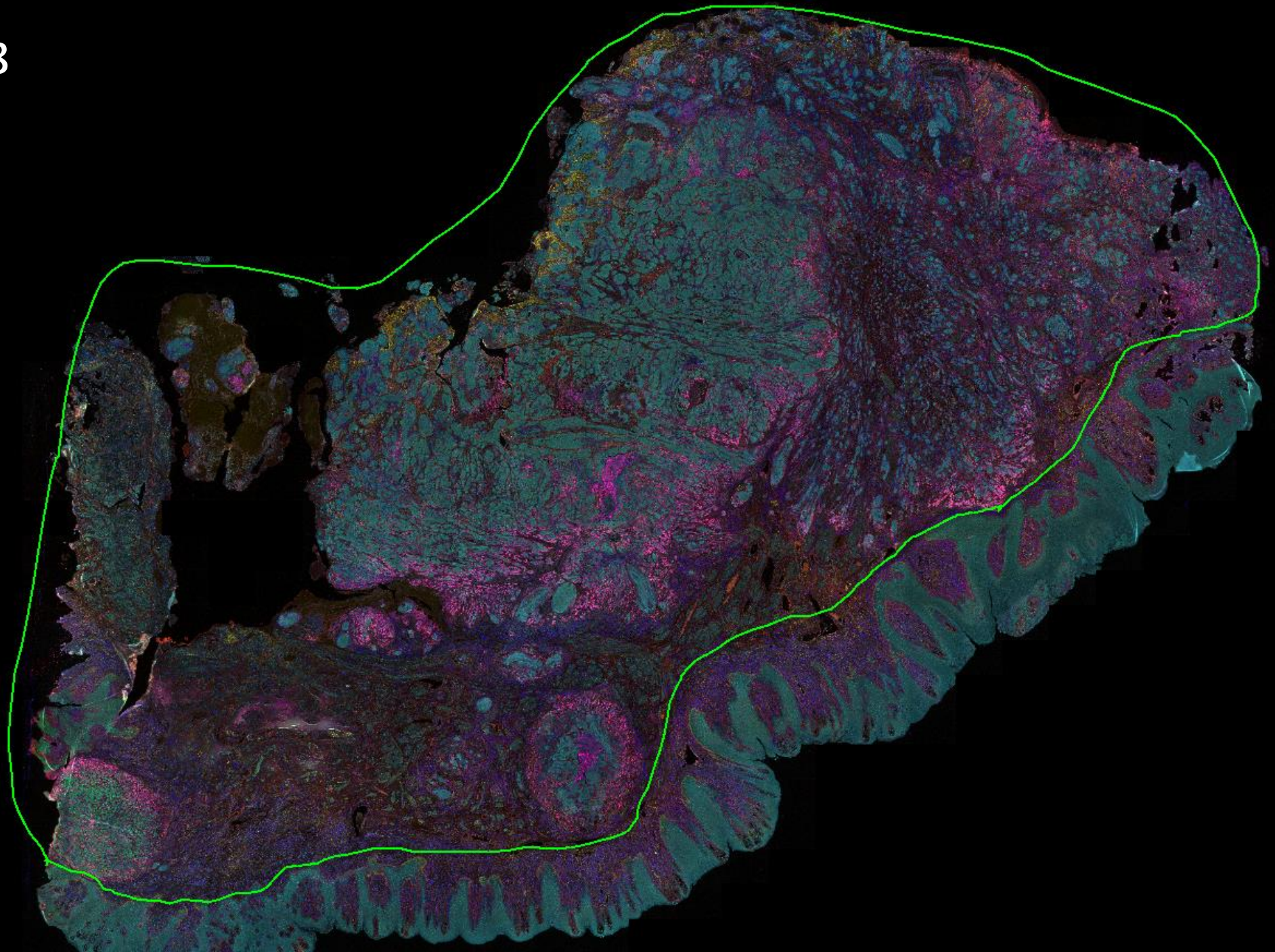
Linda Nieman, PhD

HN18

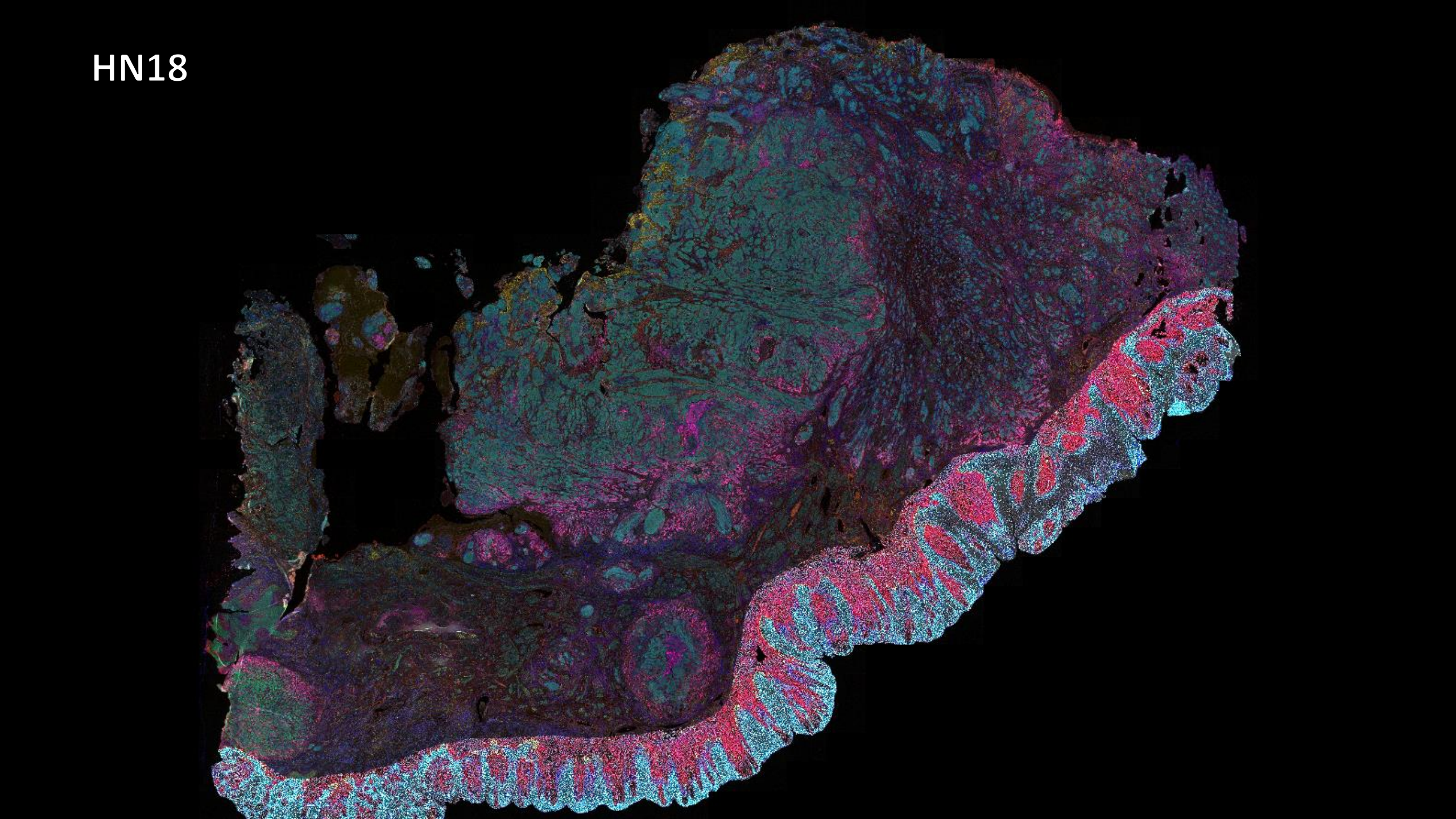


HN18

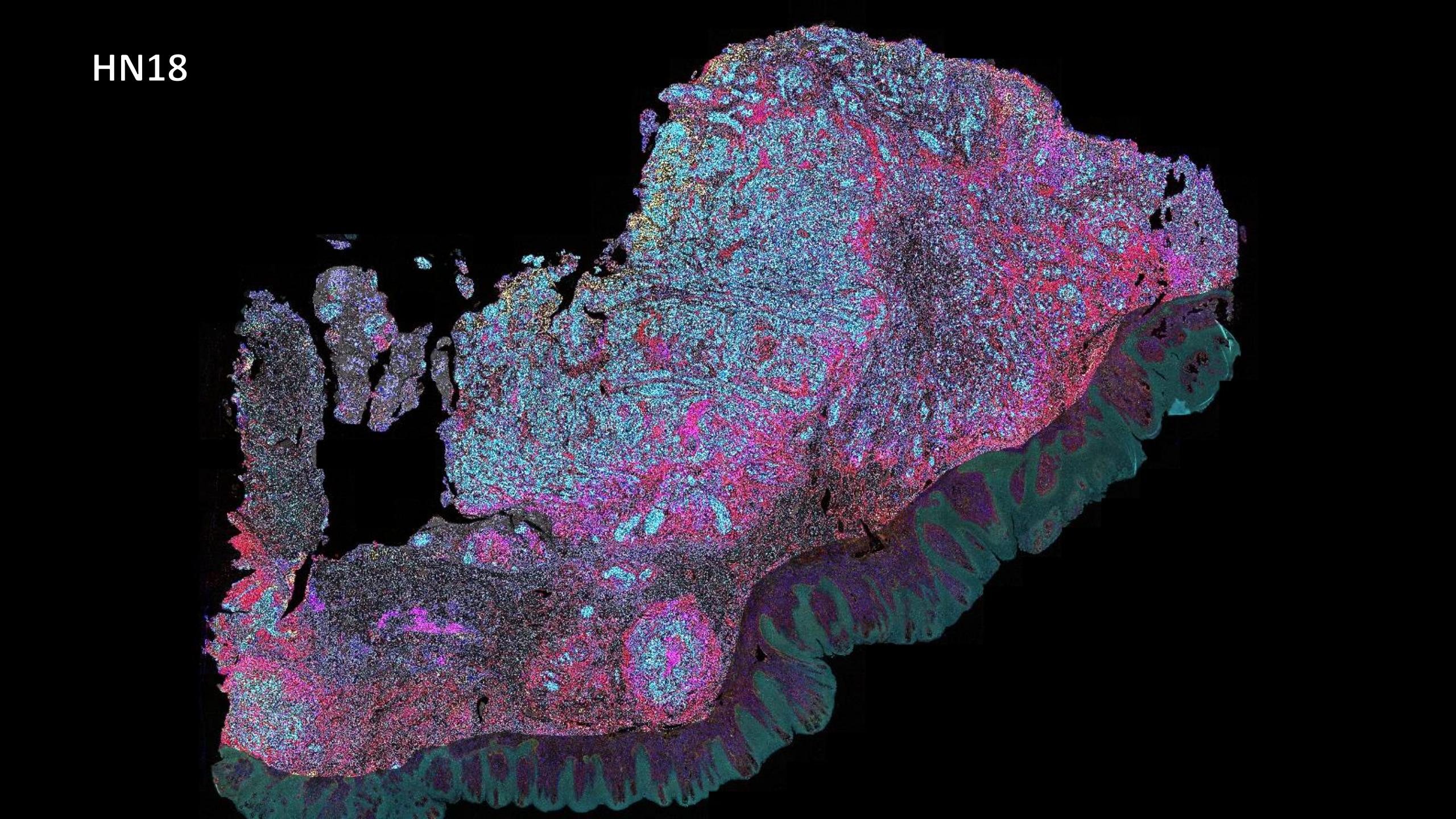
HN18

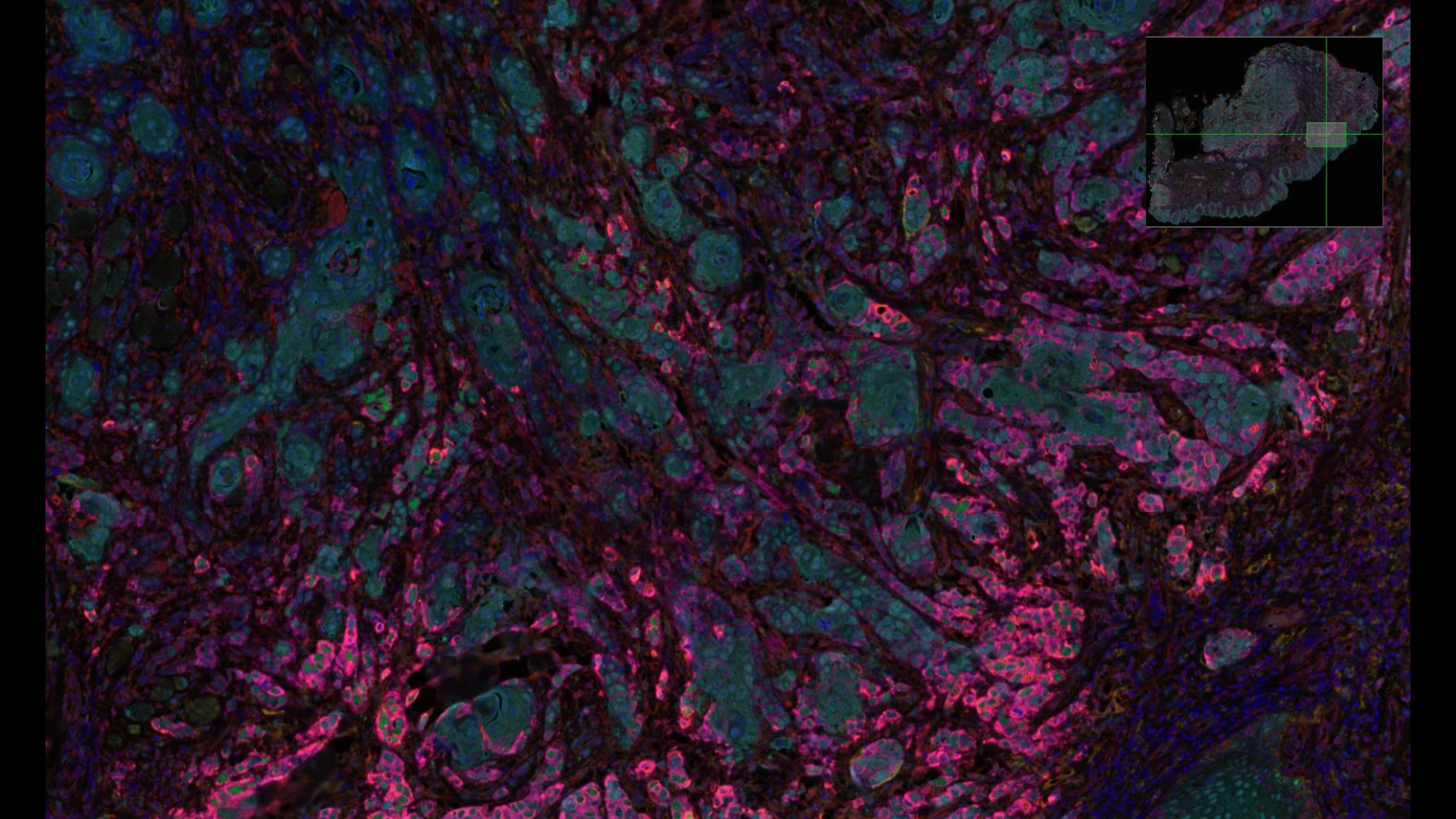


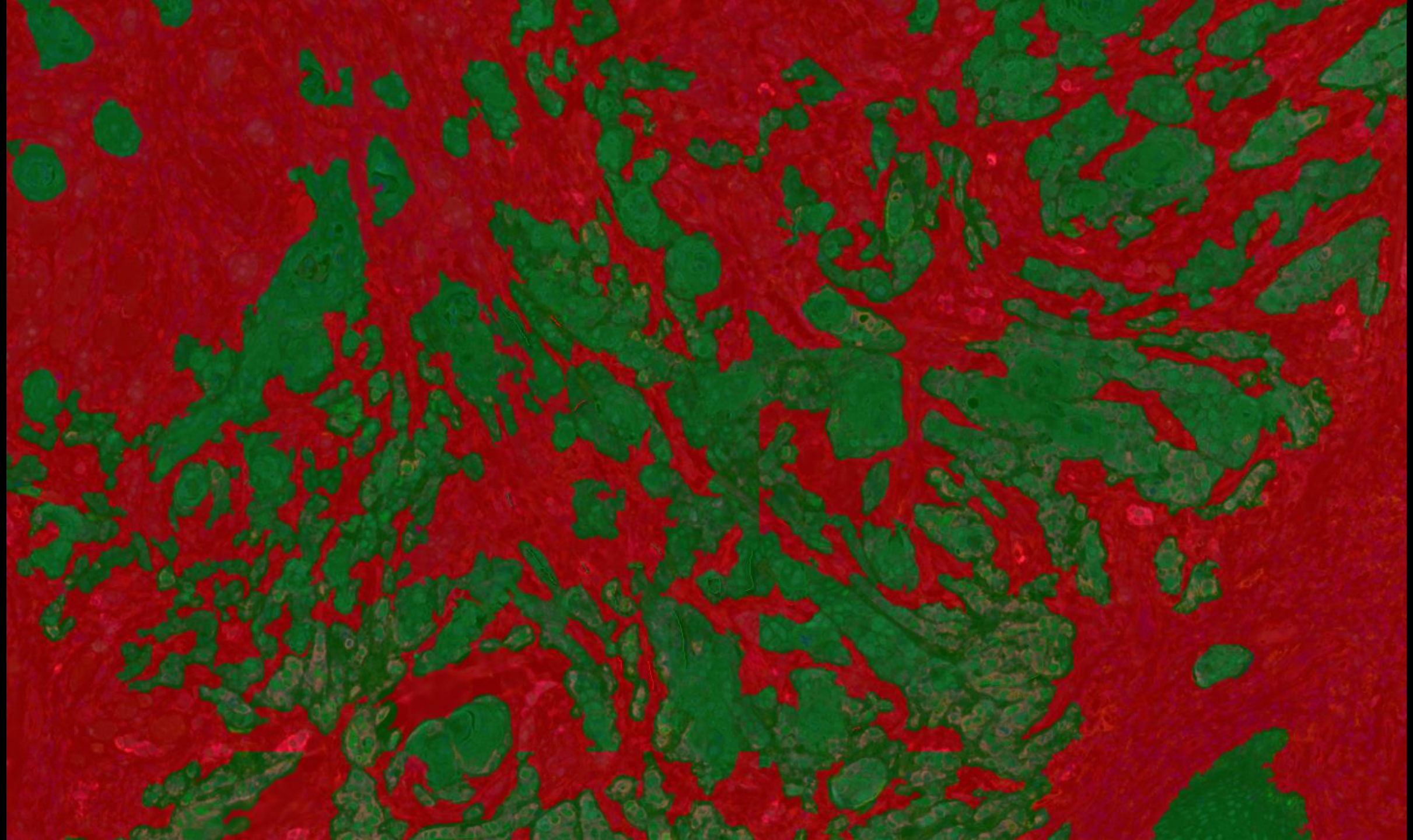
HN18

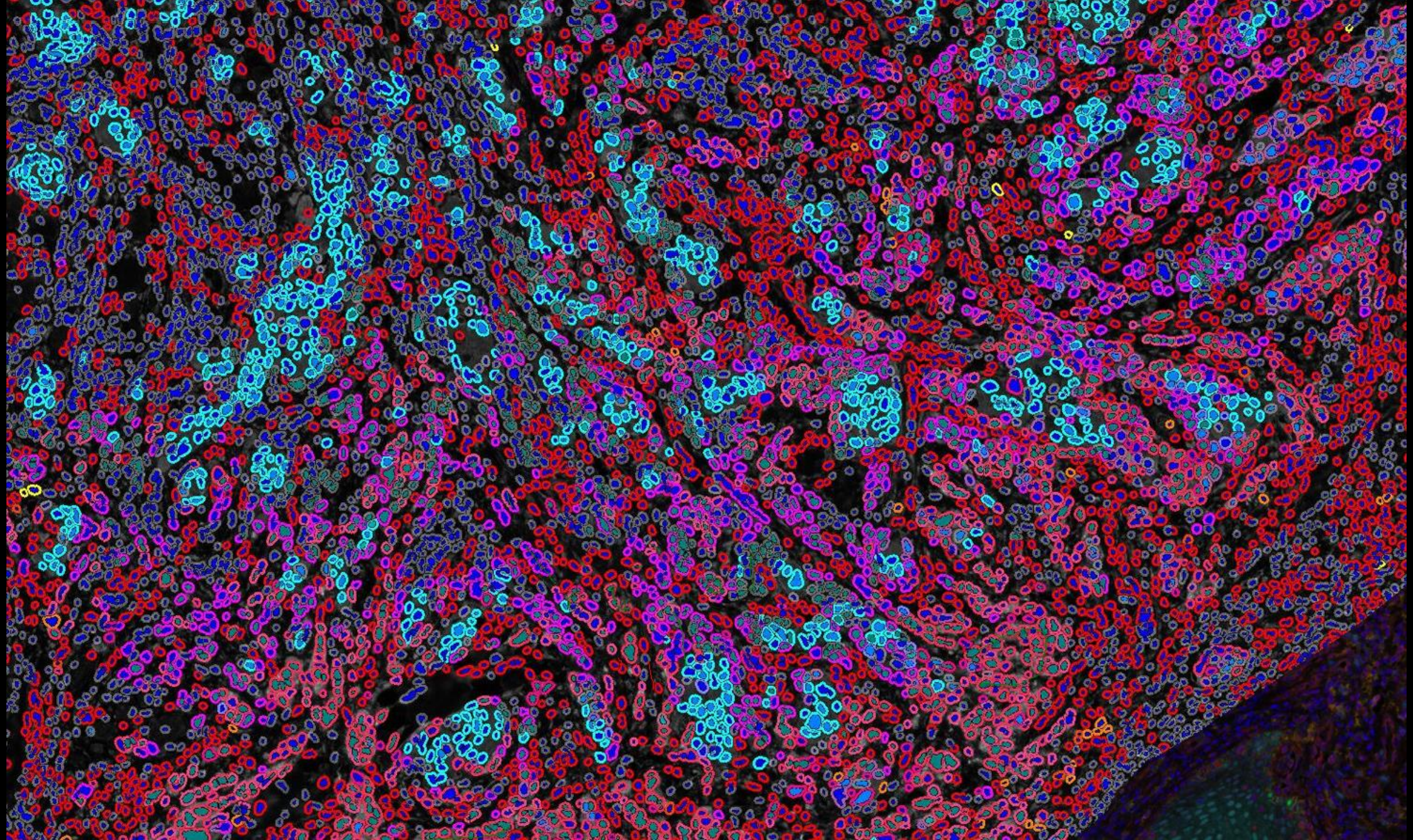


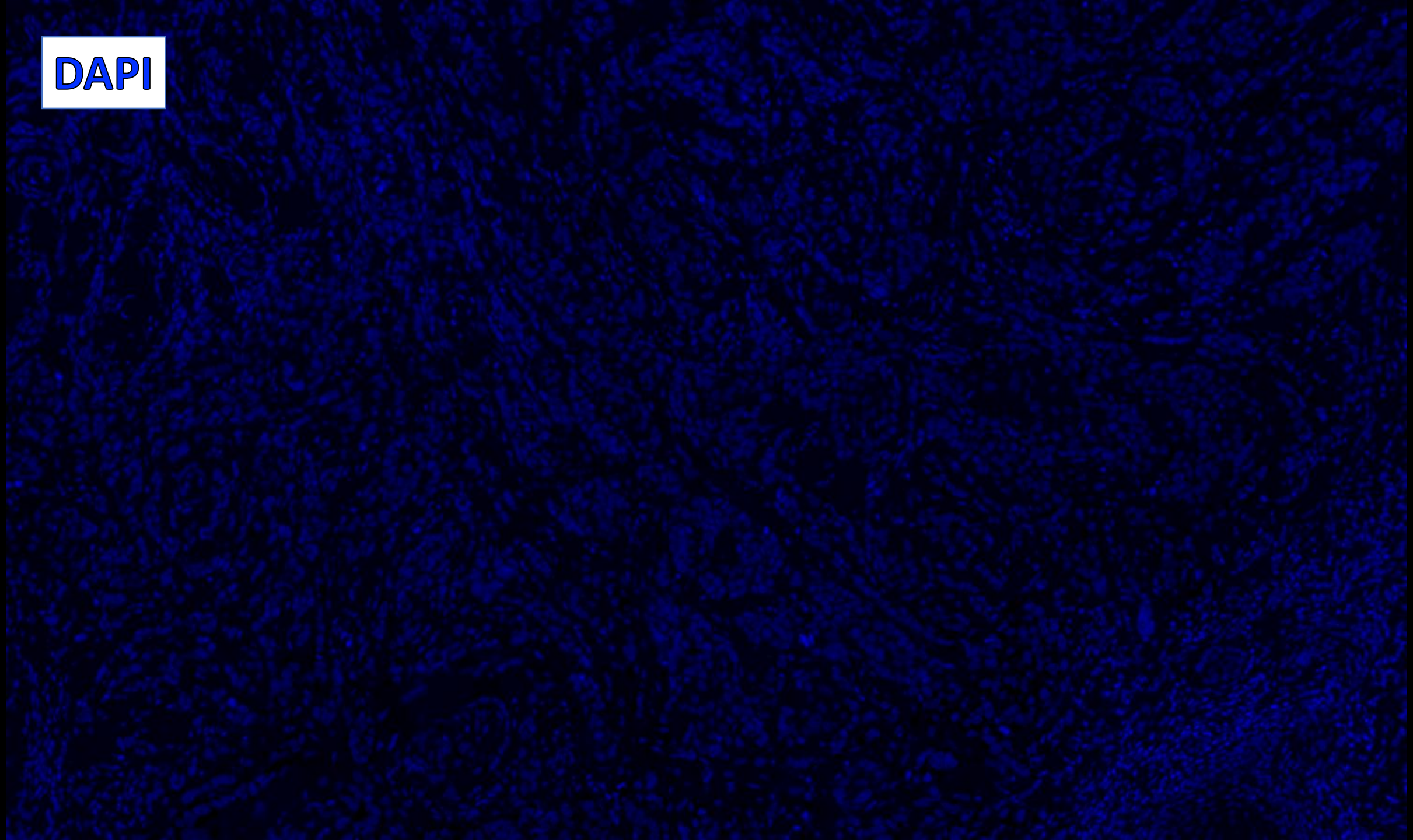
HN18

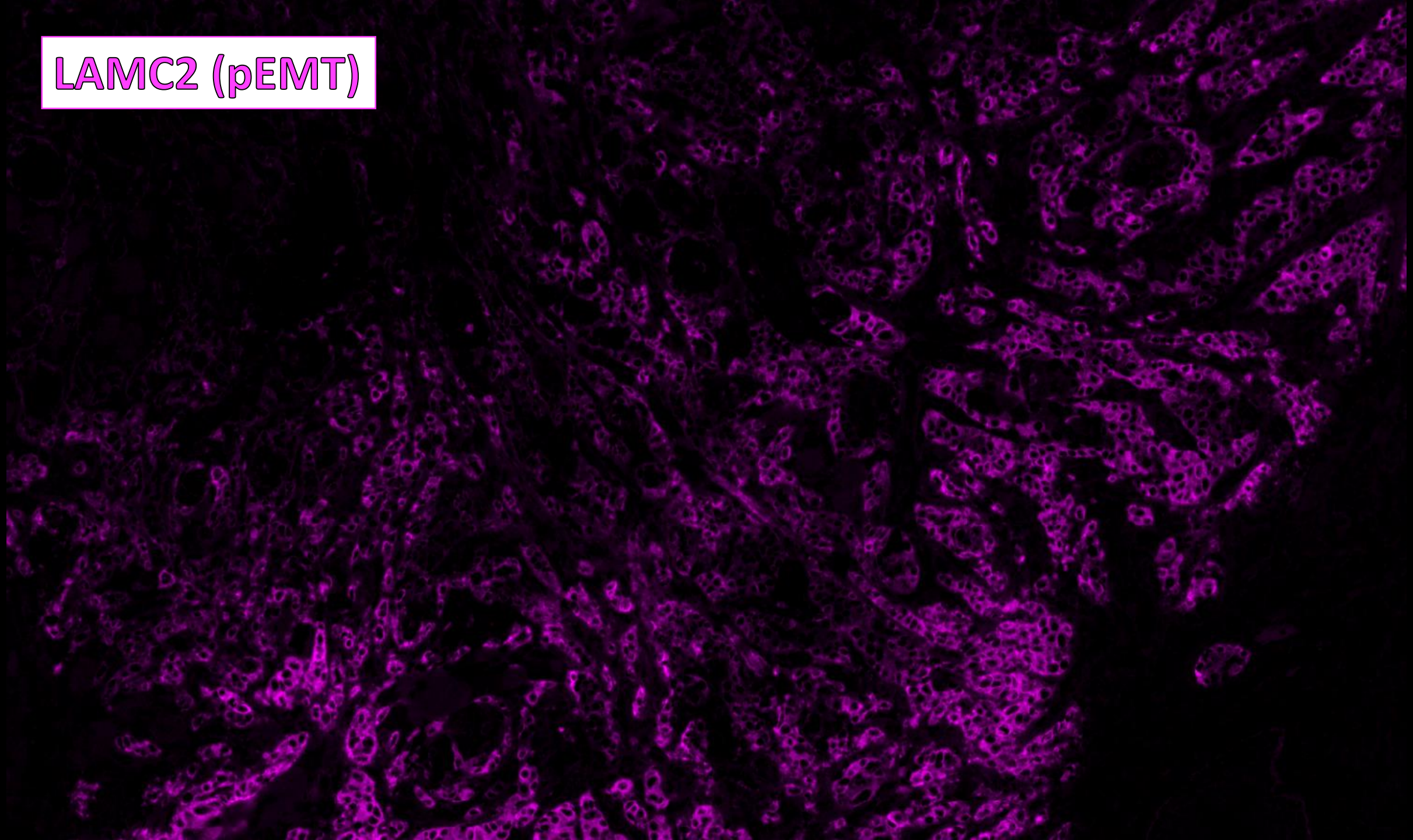


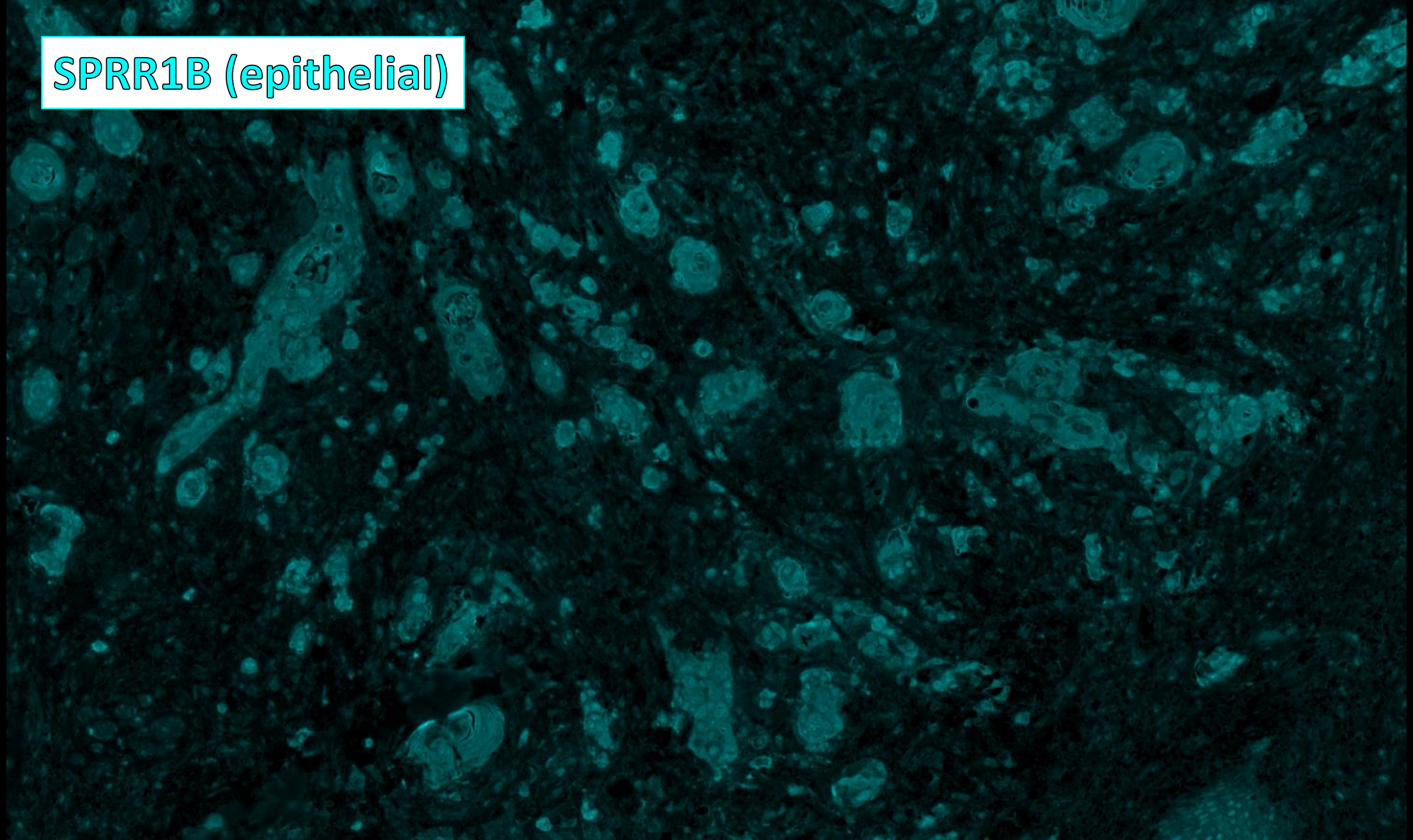




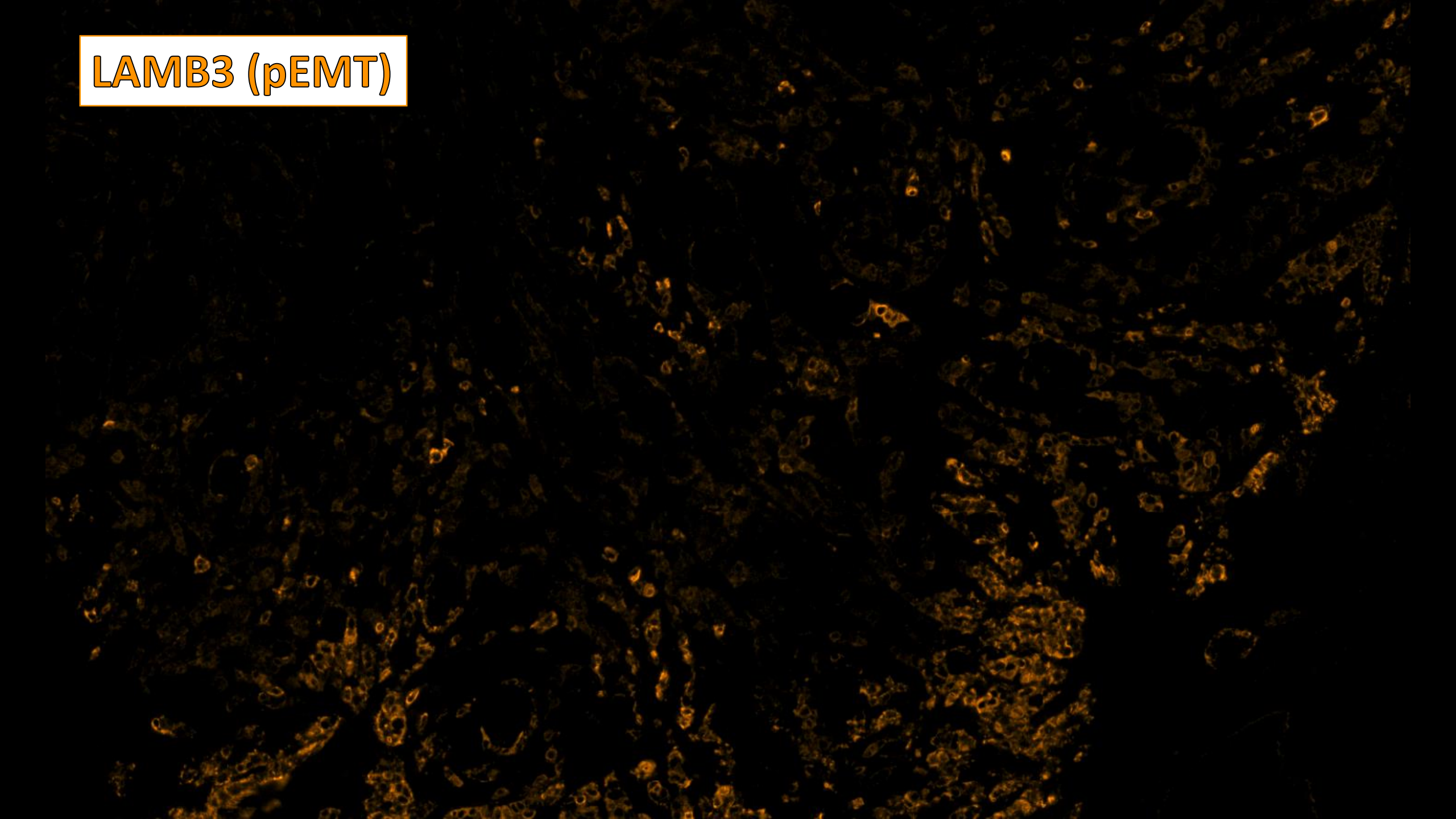


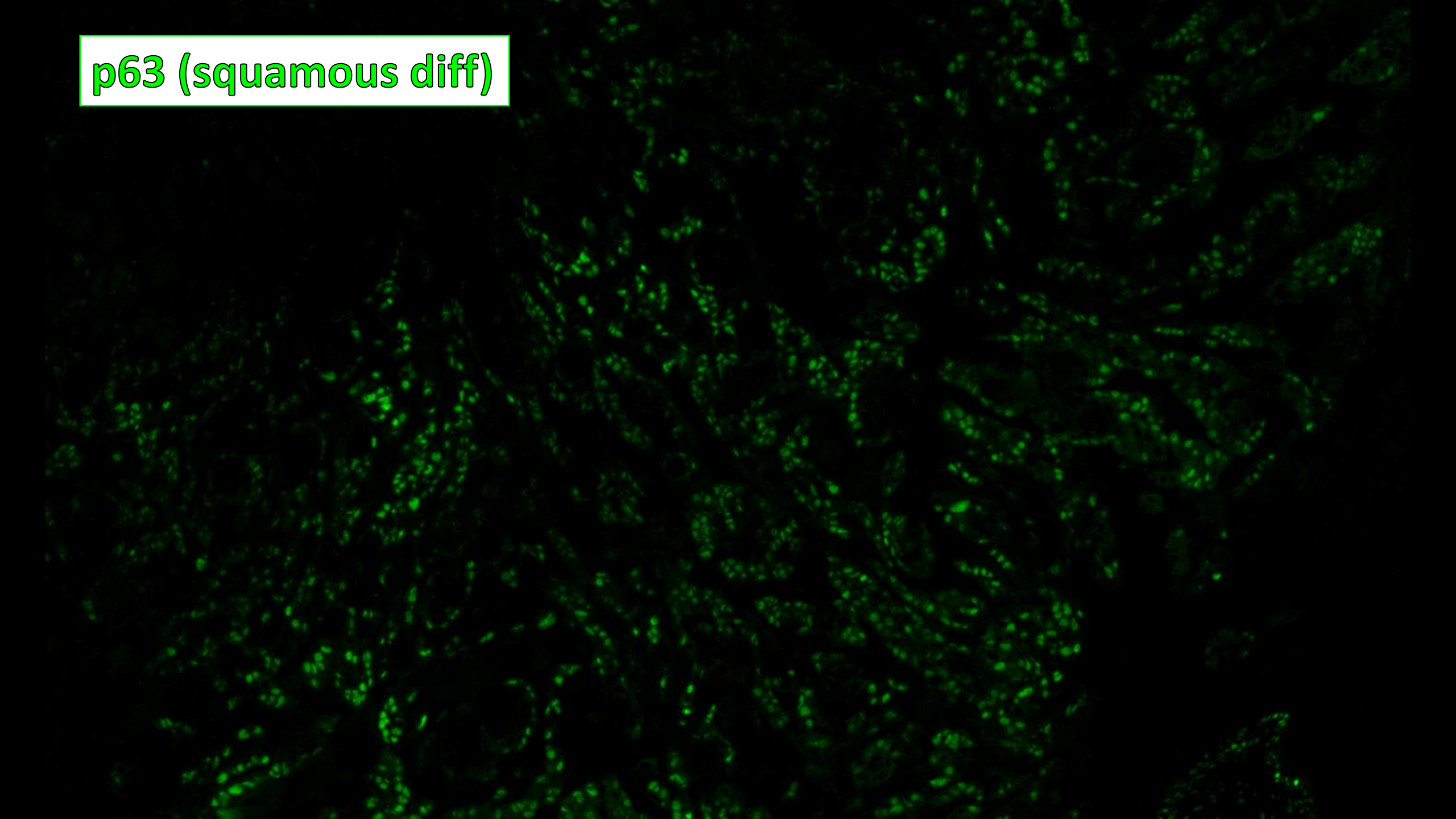

DAPI


LAMC2 (pEMT)

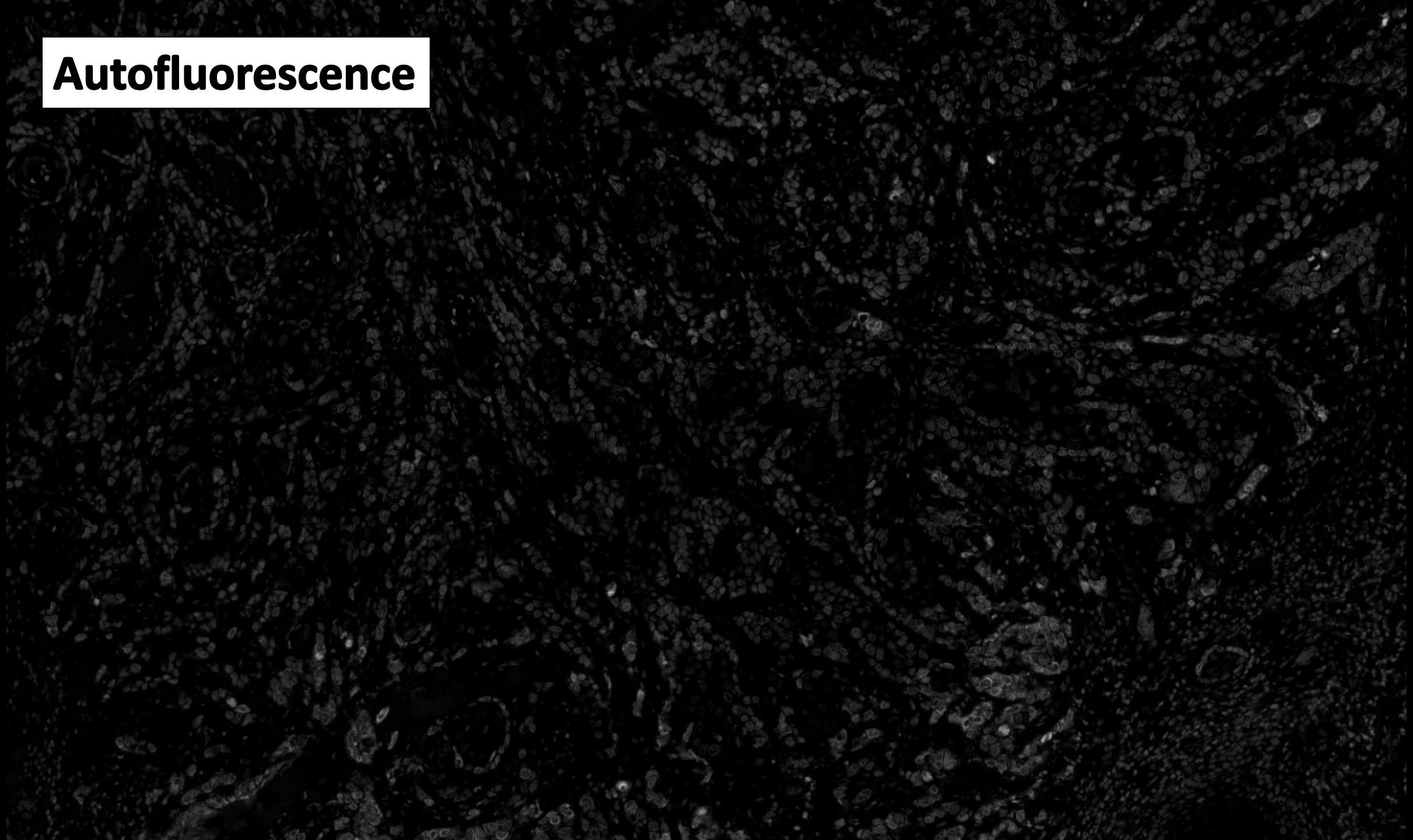

SPRR1B (epithelial)

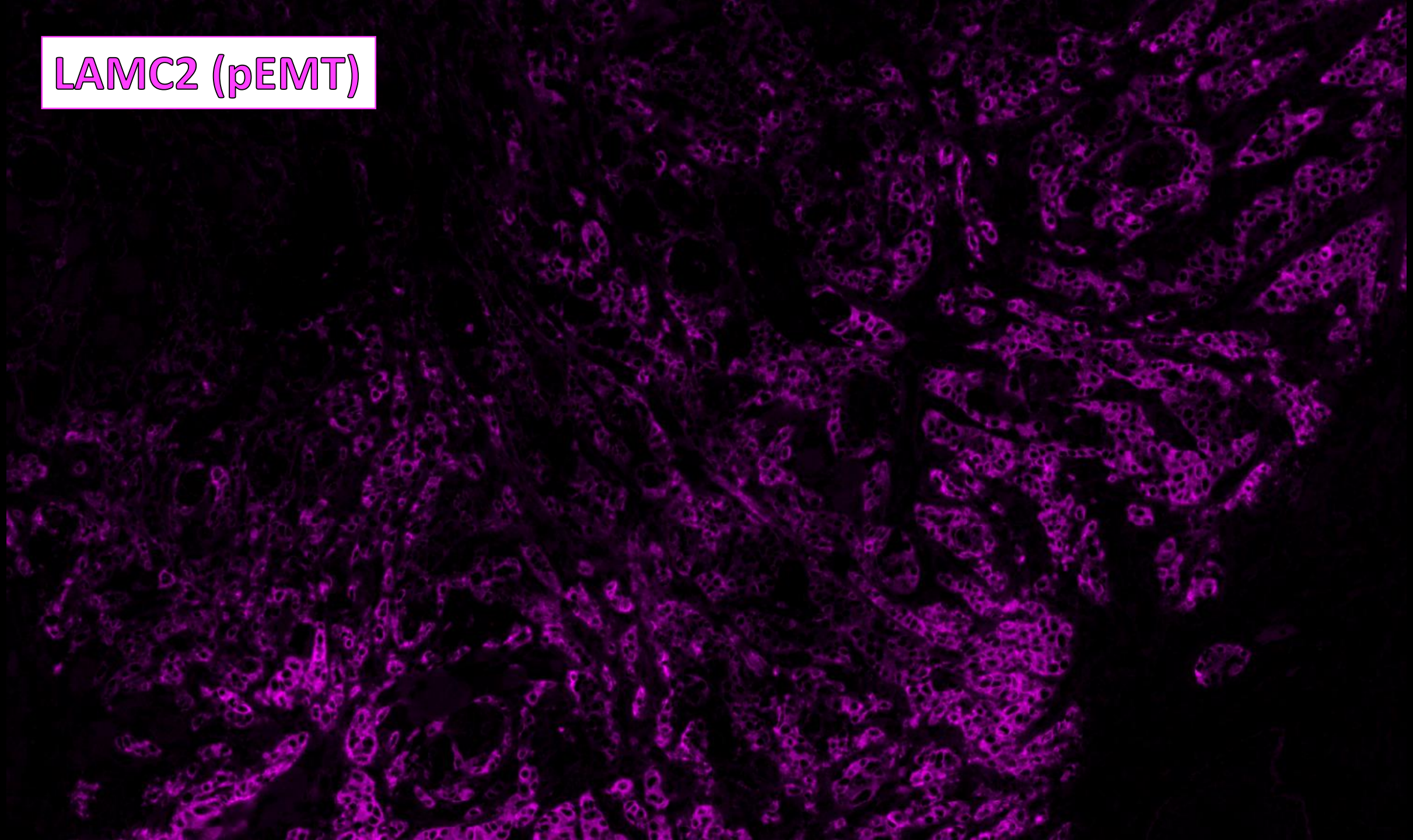
VIM (pEMT/EMT)

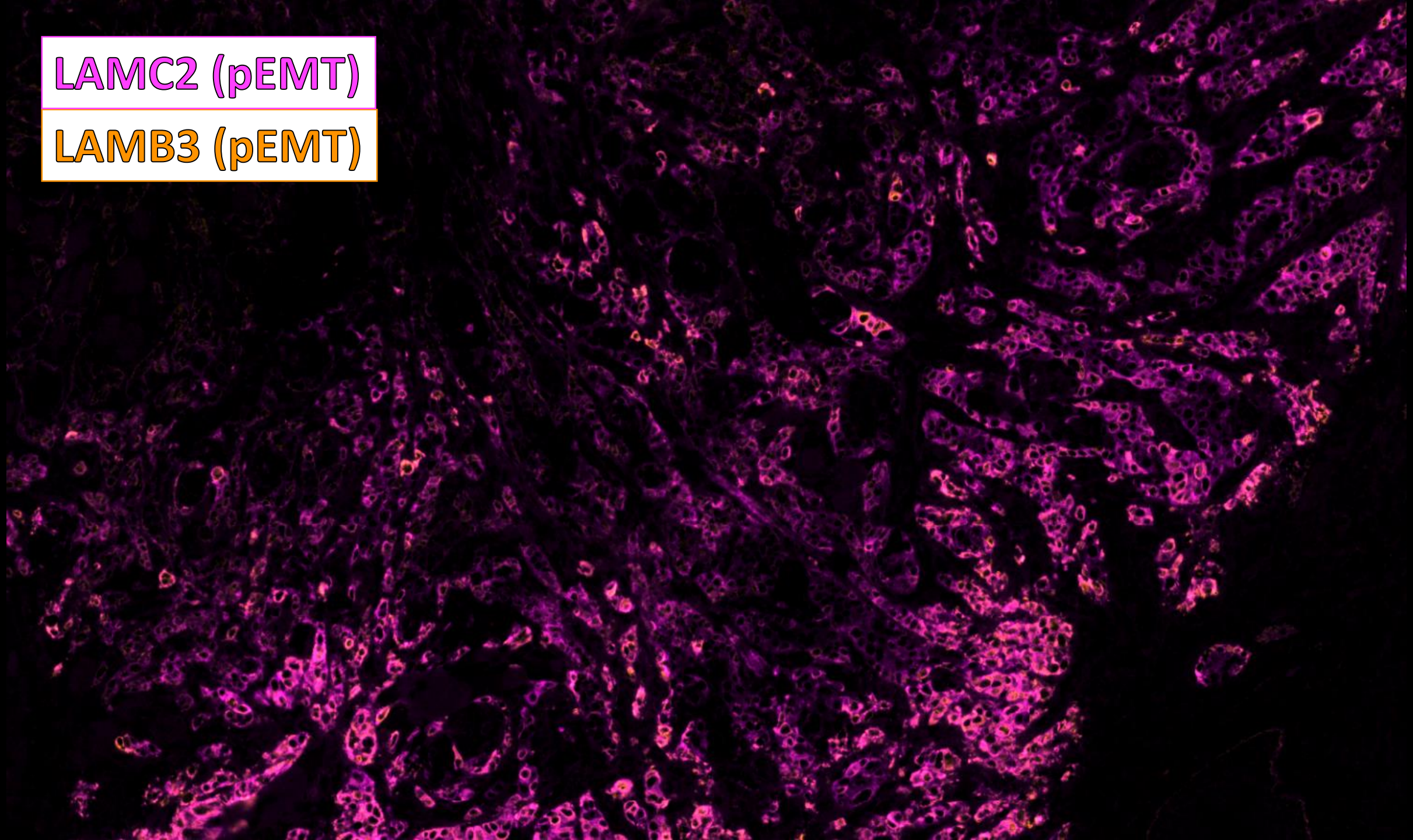

LAMB3 (pEMT)

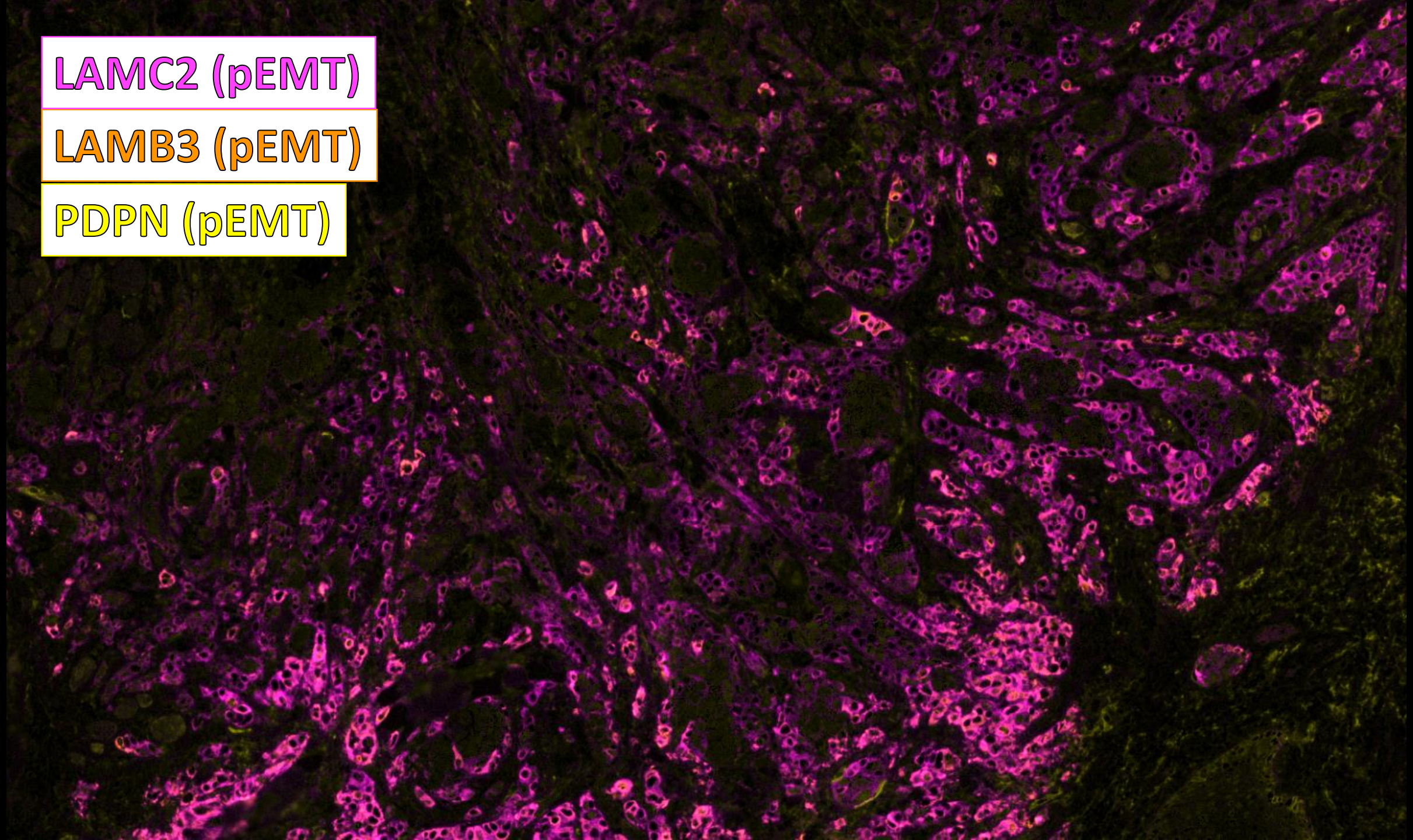

p63 (squamous diff)

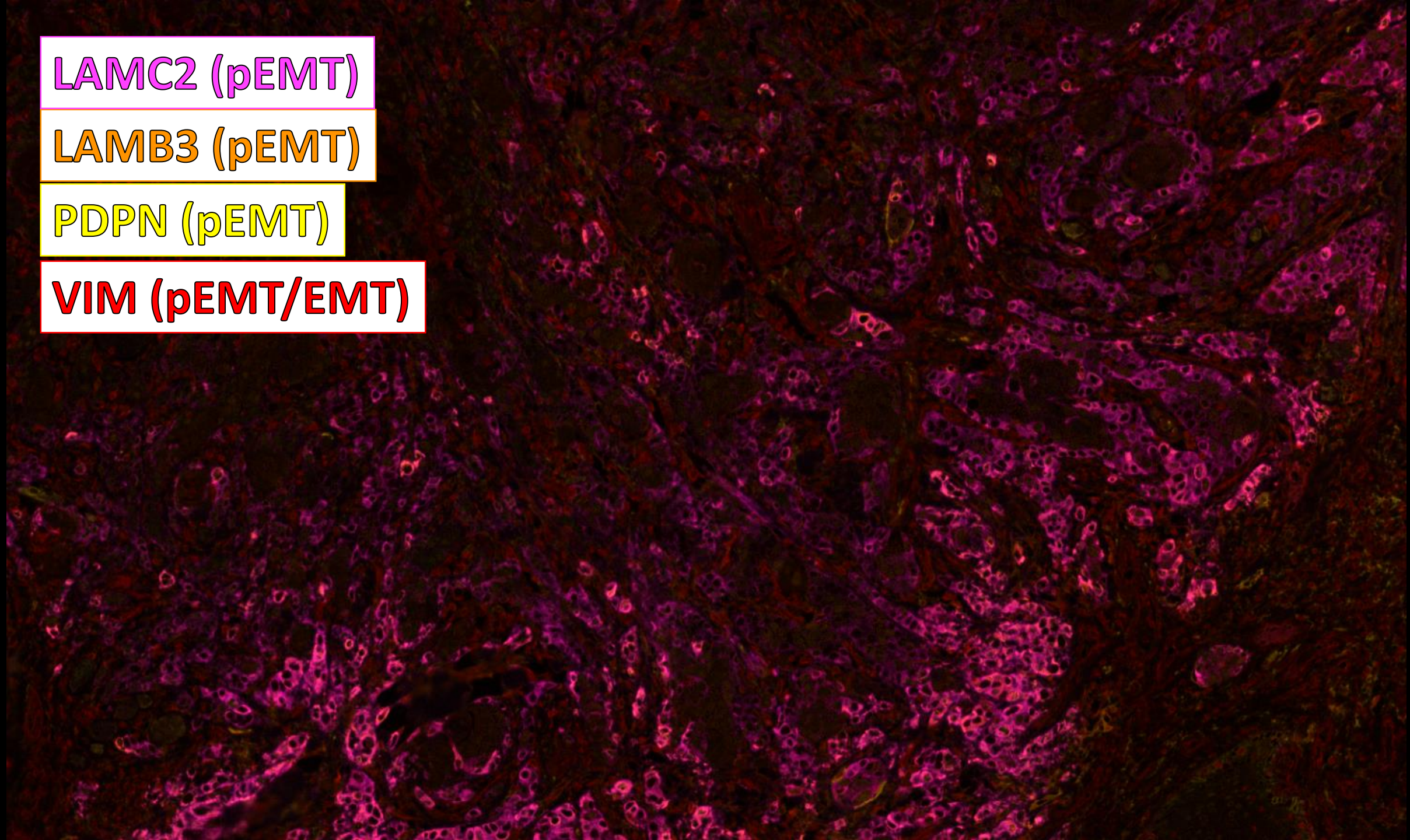

PDPN (pEMT)

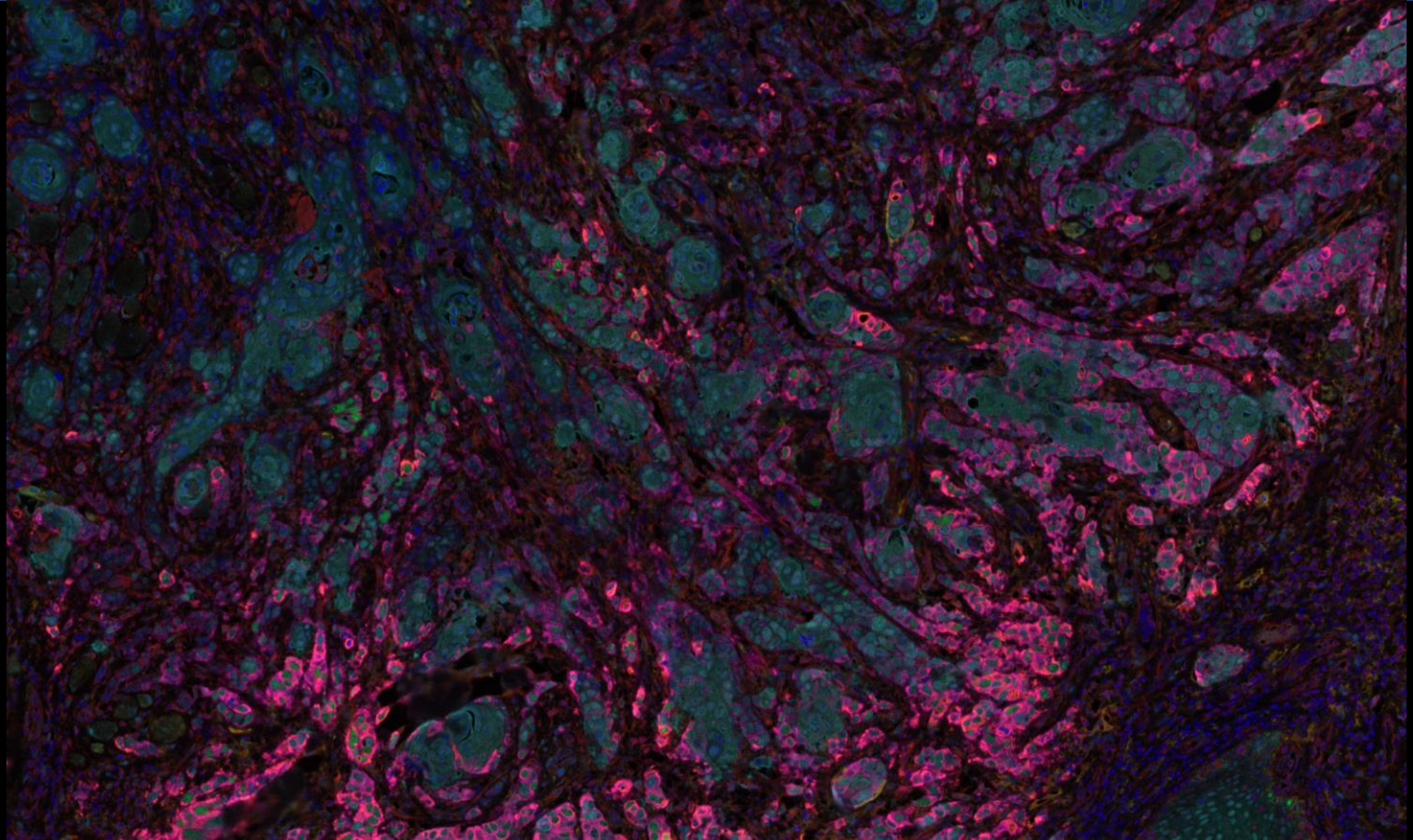

Autofluorescence


LAMC2 (pEMT)


LAMC2 (pEMT)
LAMB3 (pEMT)


LAMC2 (pEMT)
LAMB3 (pEMT)
PDPN (pEMT)


LAMC2 (pEMT)
LAMB3 (pEMT)
PDPN (pEMT)
VIM (pEMT/EMT)

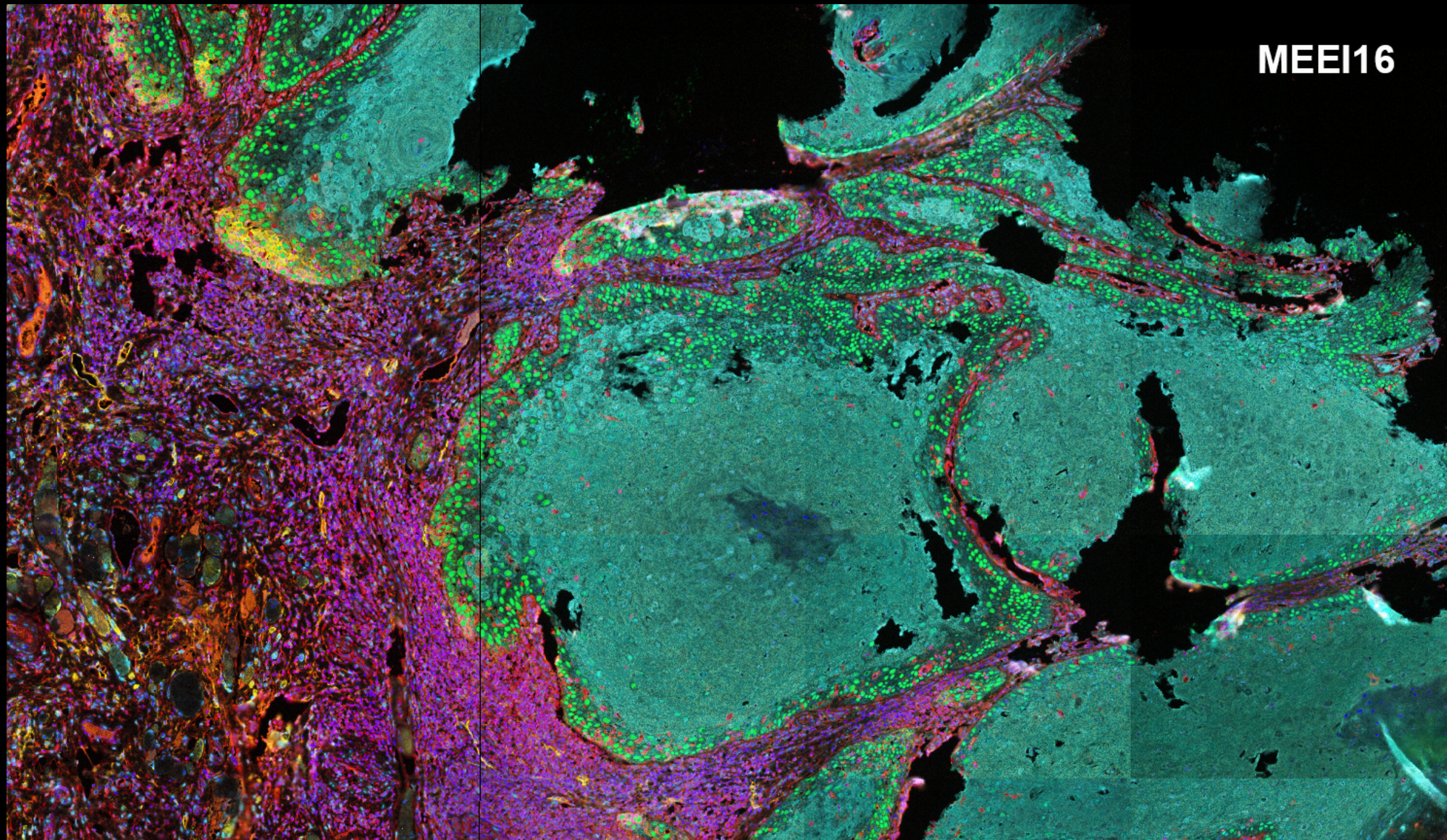


# Head and Neck MSI Tissue Staining

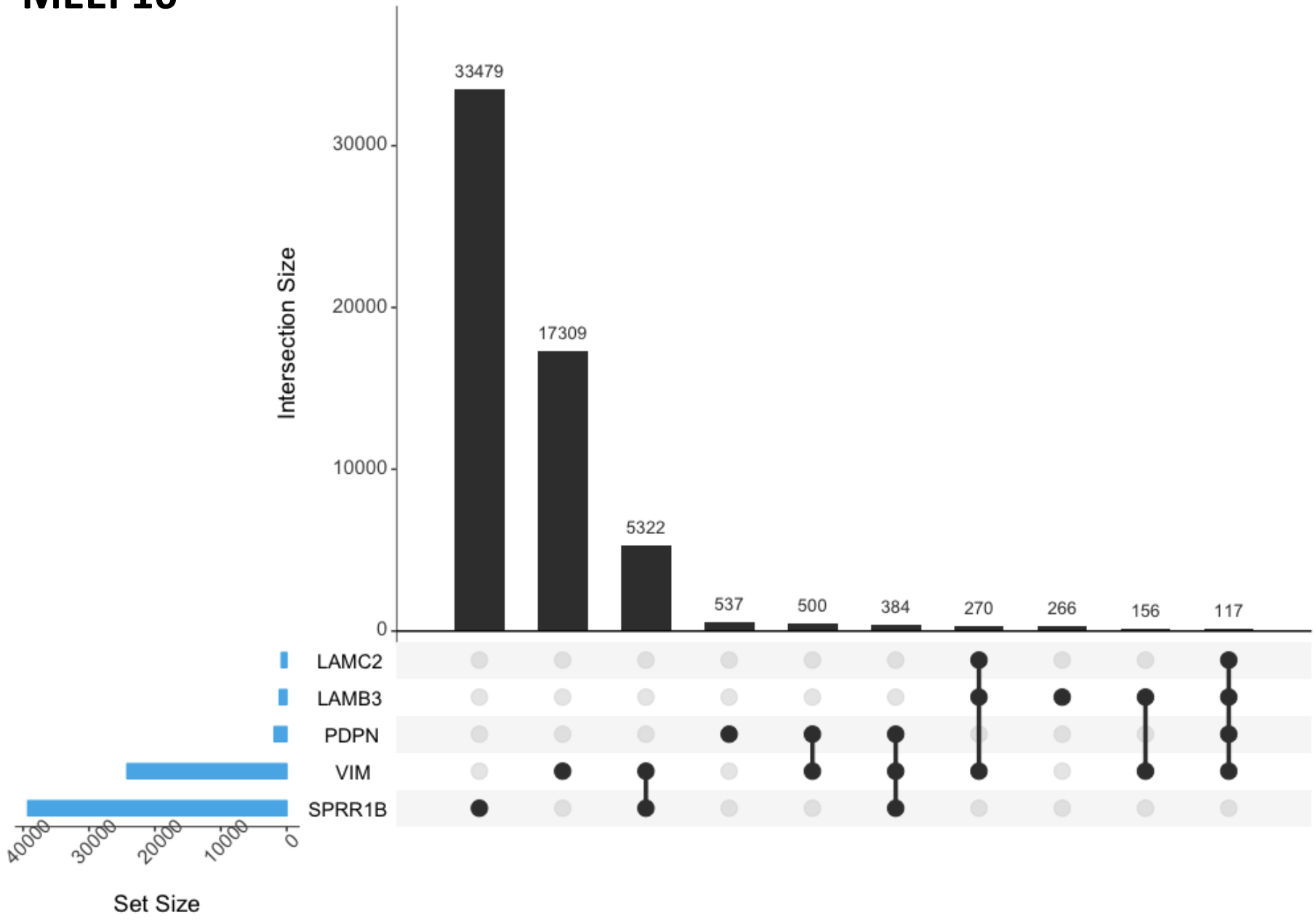
| Epithelial differentiation | p-EMT | Tumor |
| --- | --- | --- |
| SPRR1B | LAMC2 | p63 |
| | LAMB3 | |
| | VIM | |
| | PDPN | |

# MEEI 16

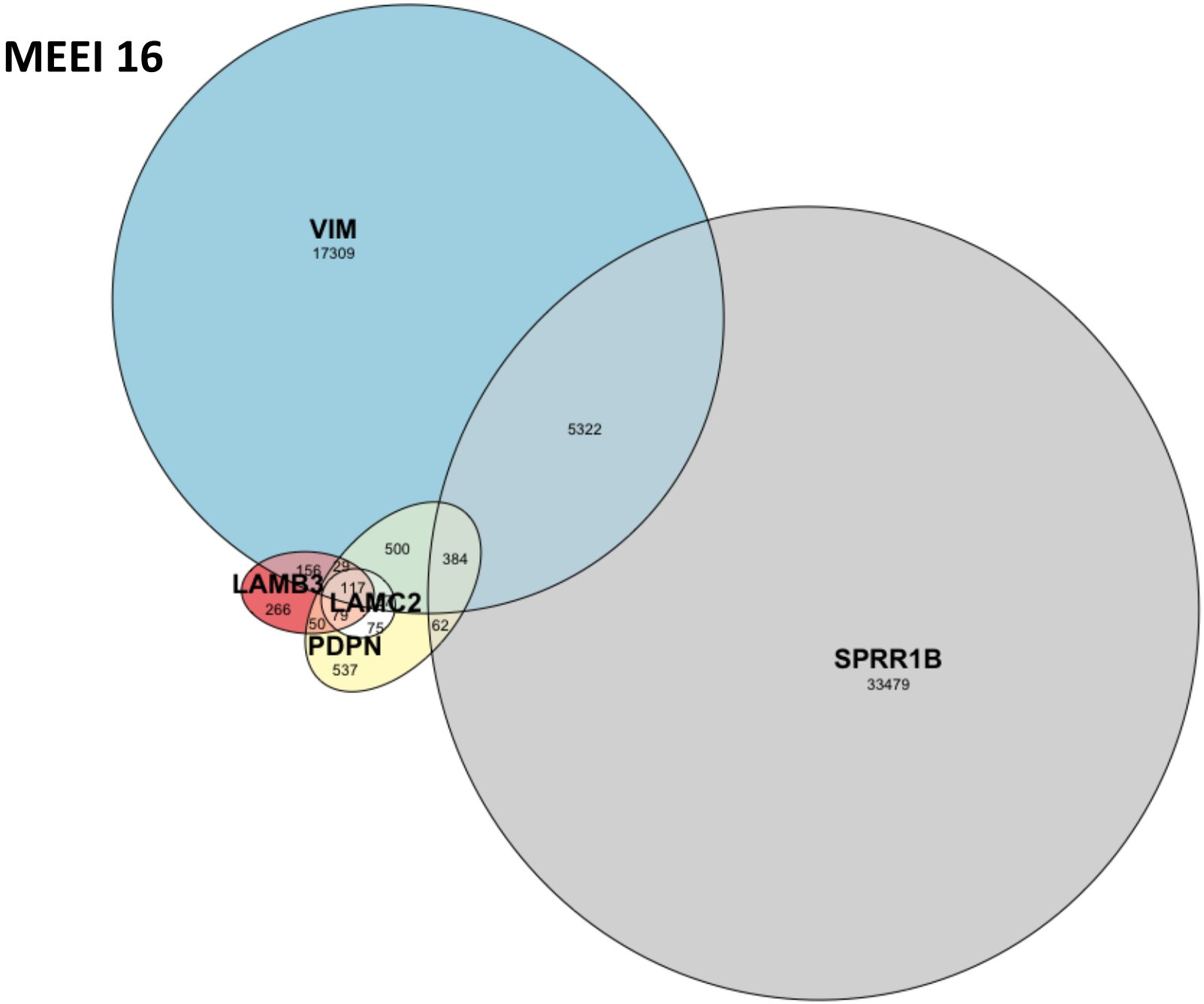
T2N0
Grade 1
(-) Perineural Invasion
(-) Lymphovascular Invasion


MEEI16

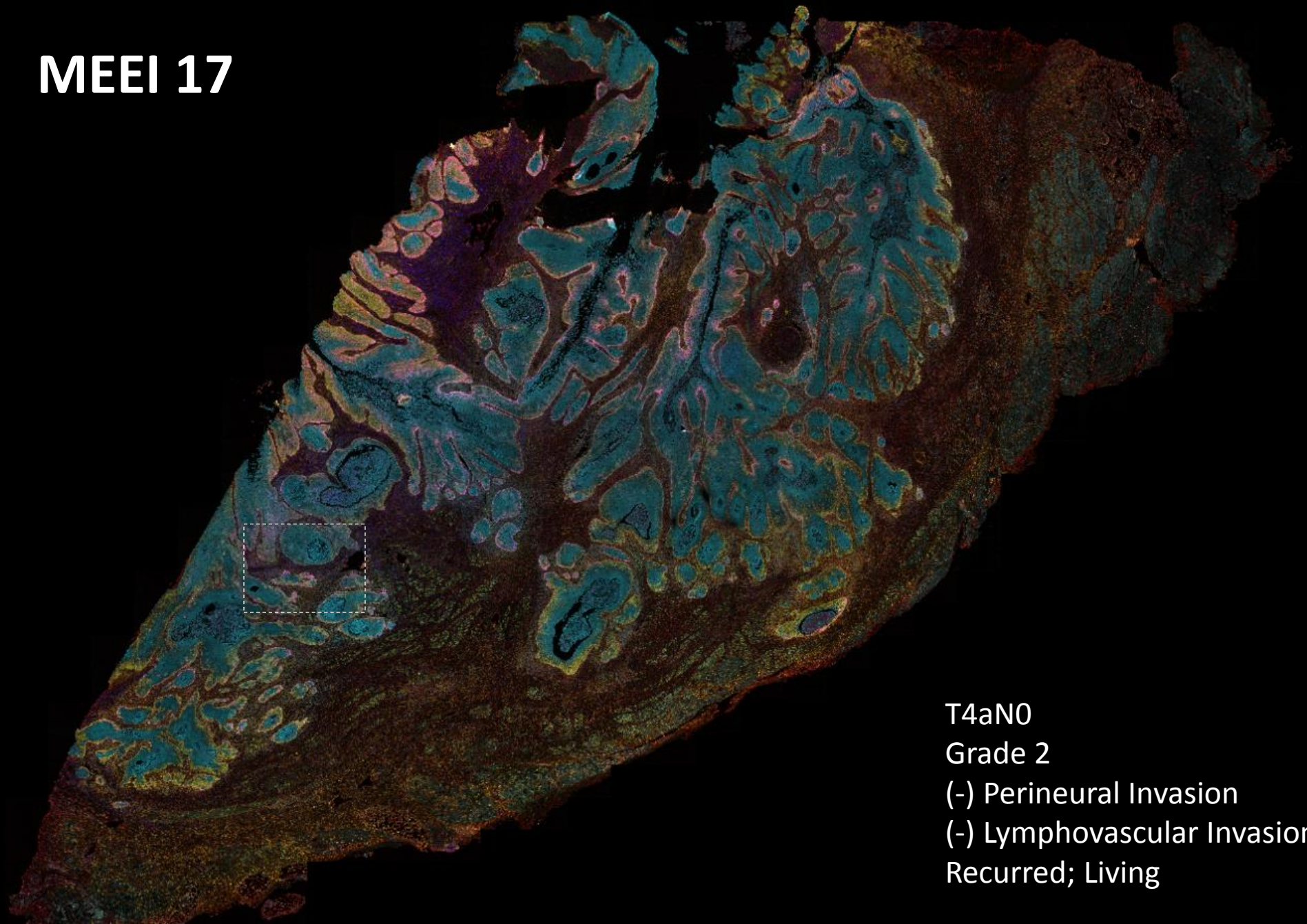
# MEEI 16


Intersection Size
33479
17309
5322
537
500
384
270
266
156
117
30000
20000
10000
0
LAMC2
LAMB3
PDPN
VIM
SPRR1B
40000
30000
20000
10000
0
Set Size

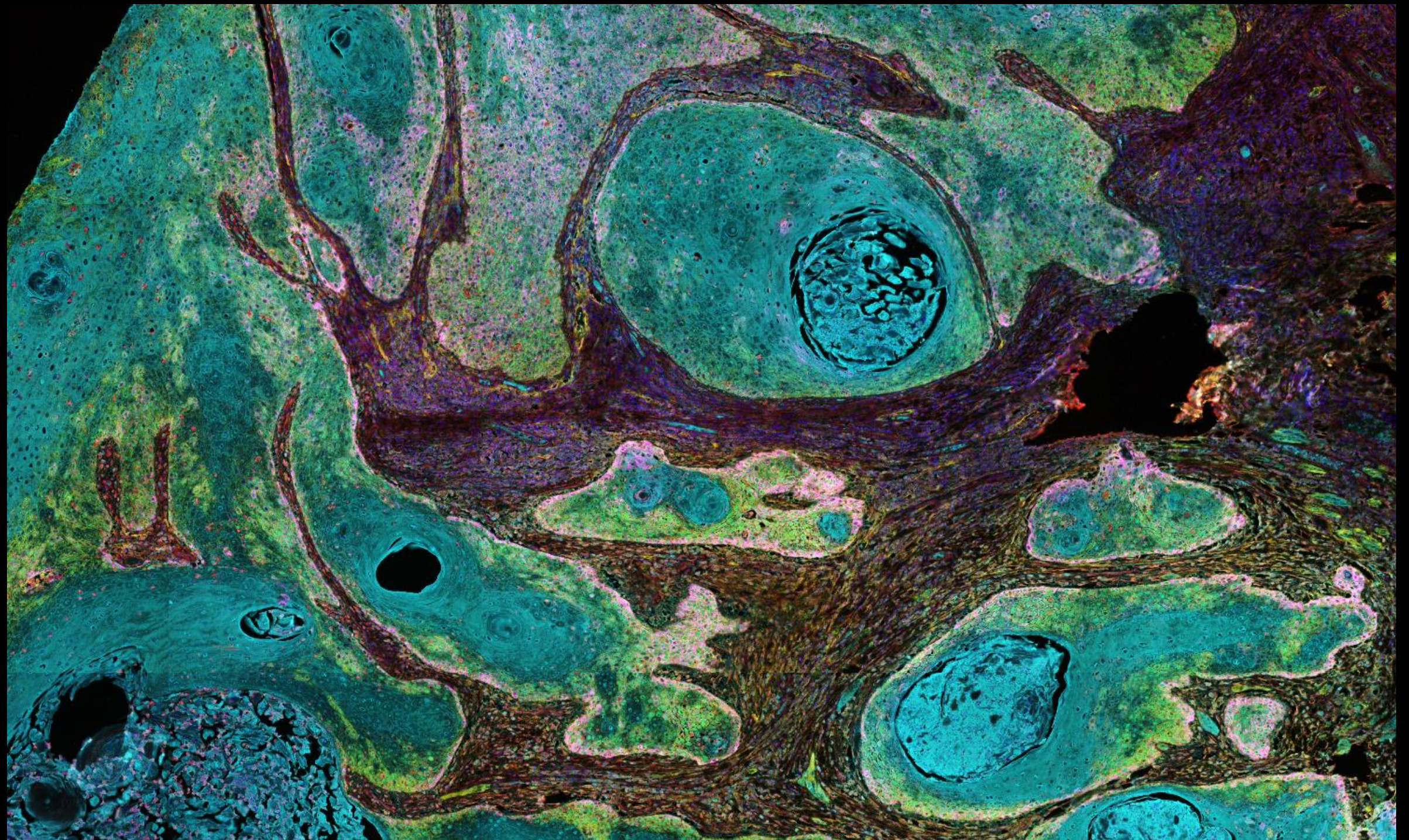
# MEEI 16

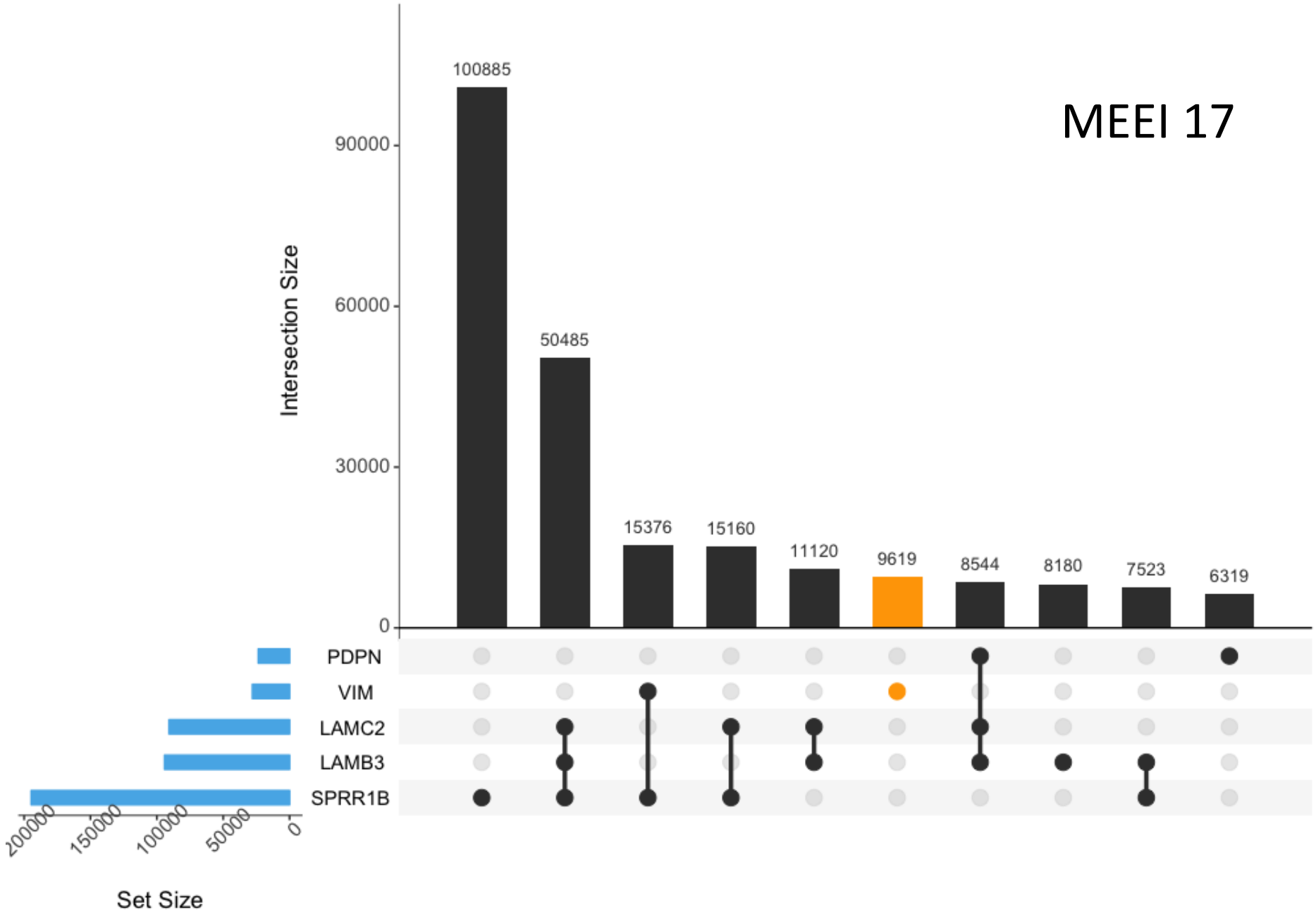

VIM
17309
5322
500
384
156
29
LAMB3
266
117
LAMC2
50
79
75
62
PDPN
537
SPRR1B
33479

# MEEI 17

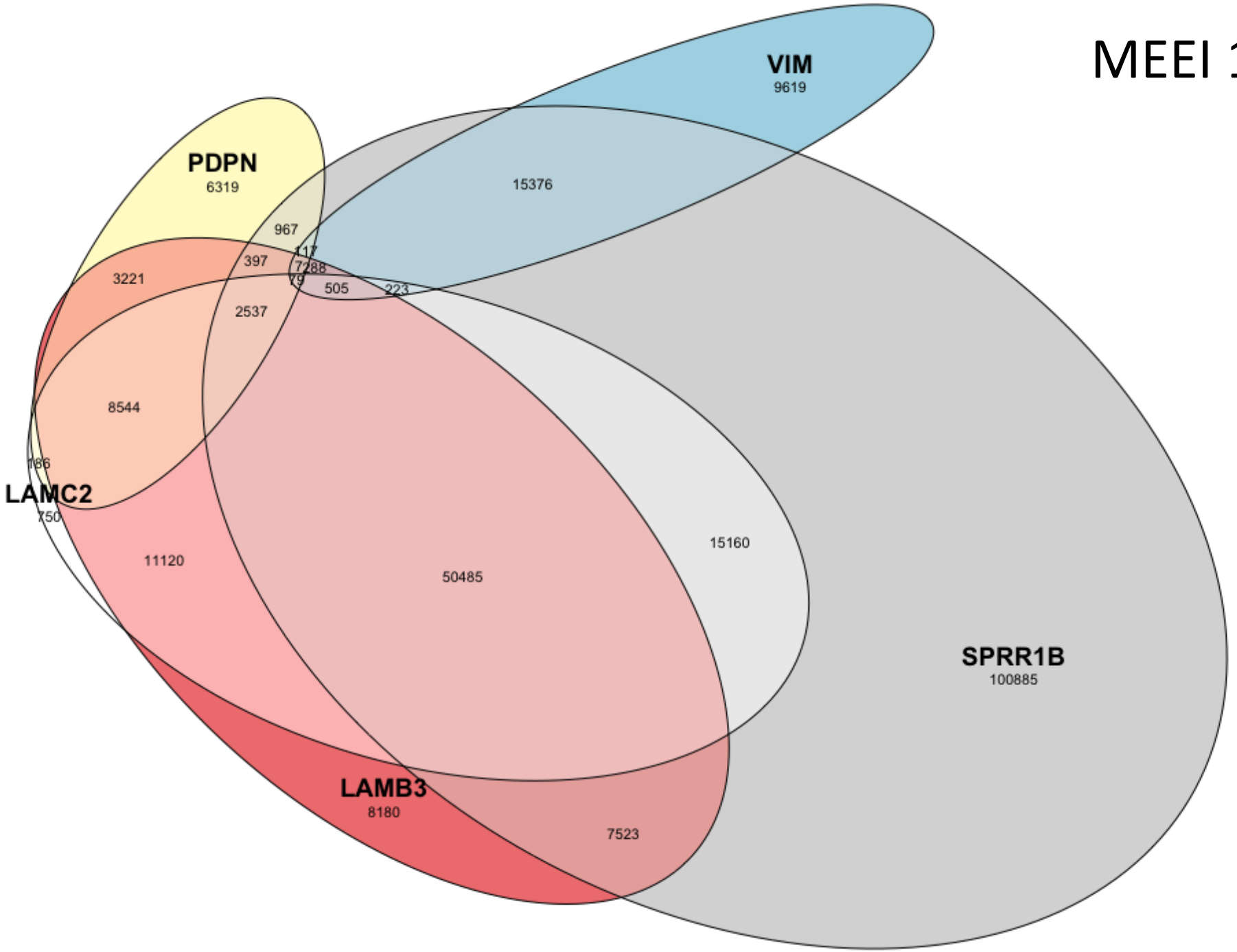


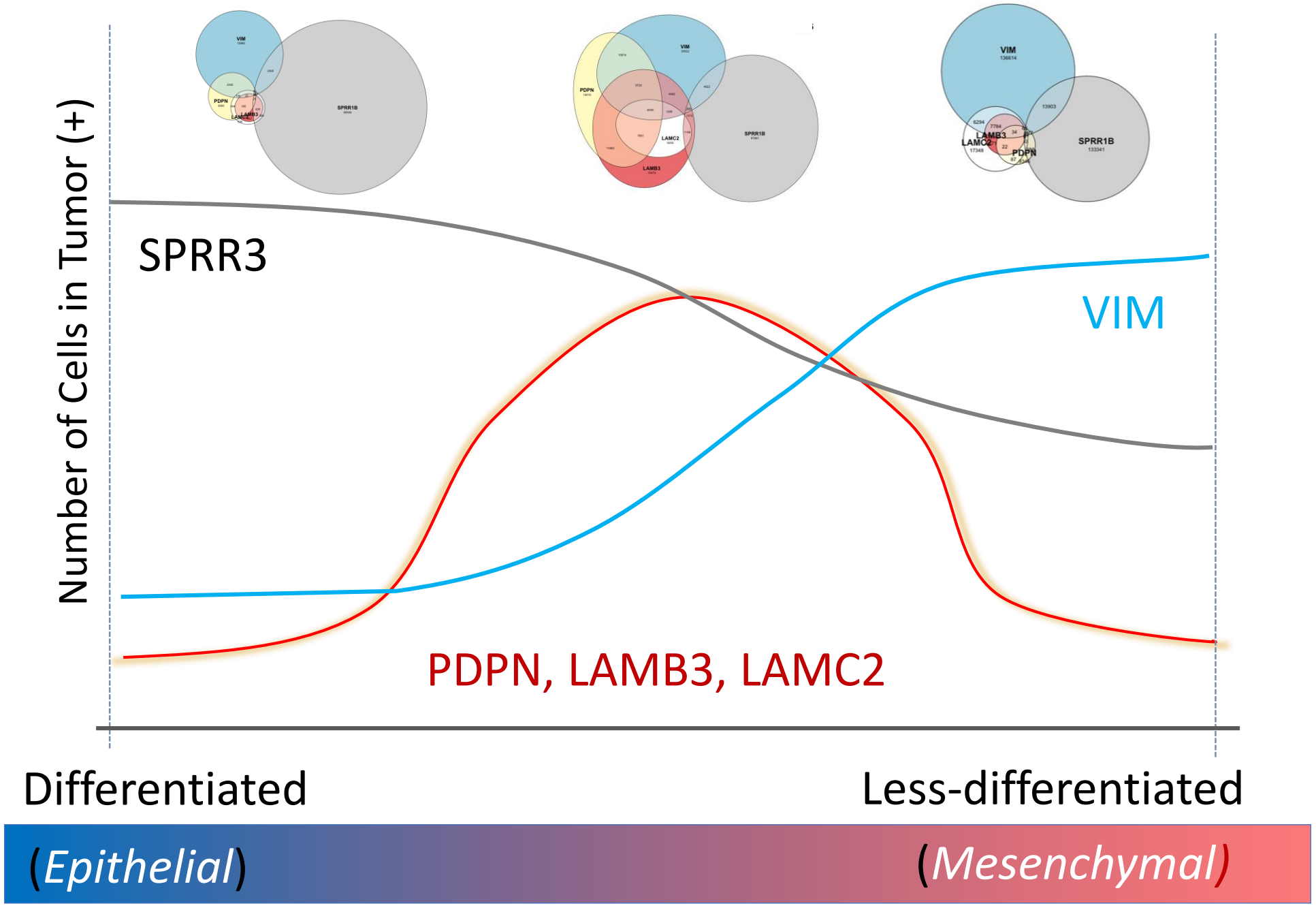
T4aN0
Grade 2
(-) Perineural Invasion
(-) Lymphovascular Invasion
Recurred; Living



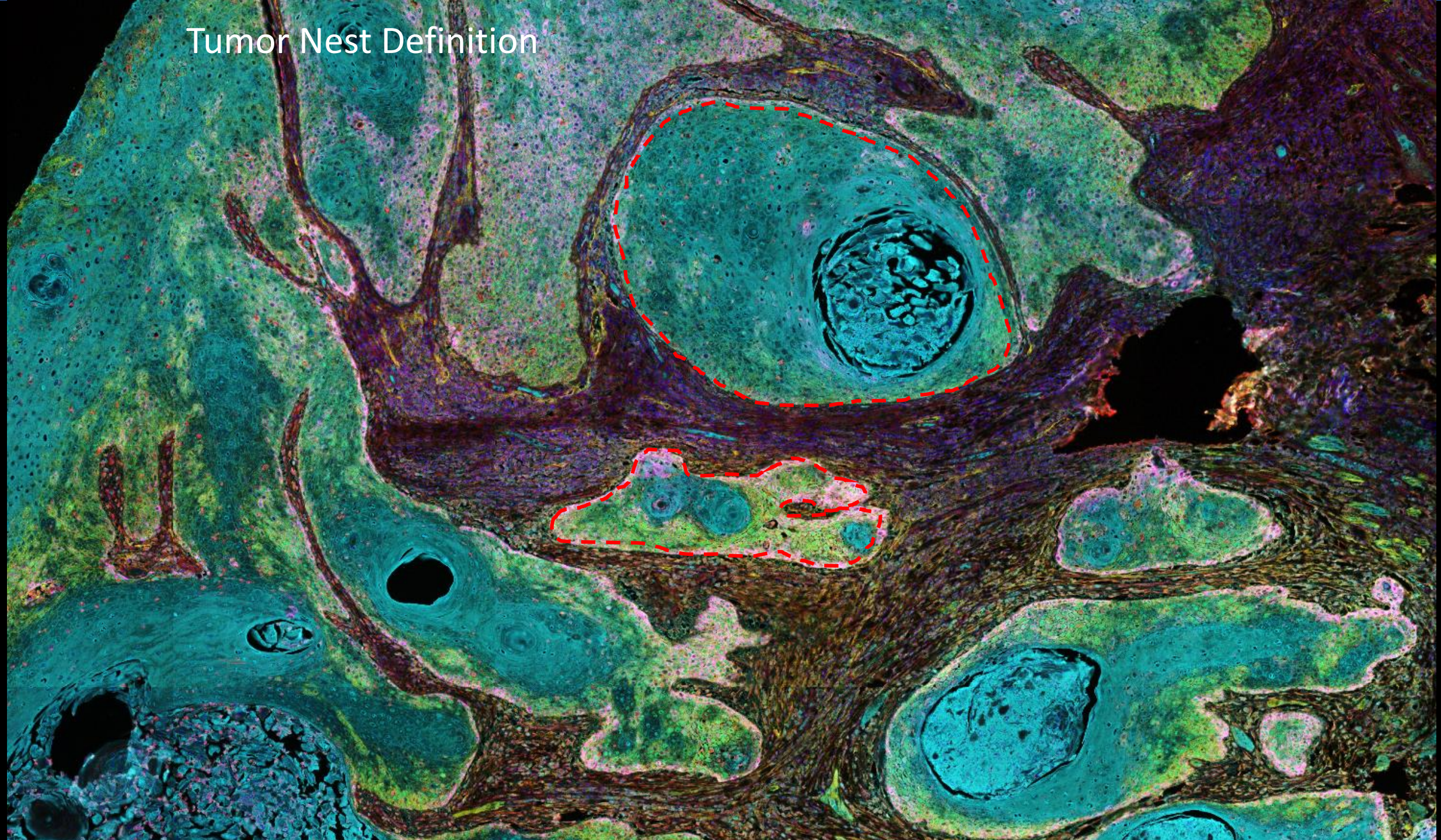

MEEI 17
Intersection Size
100885
50485
15376
15160
11120
9619
8544
8180
7523
6319
90000
60000
30000
0
PDPN
VIM
LAMC2
LAMB3
SPRR1B
200000
150000
100000
50000
0
Set Size

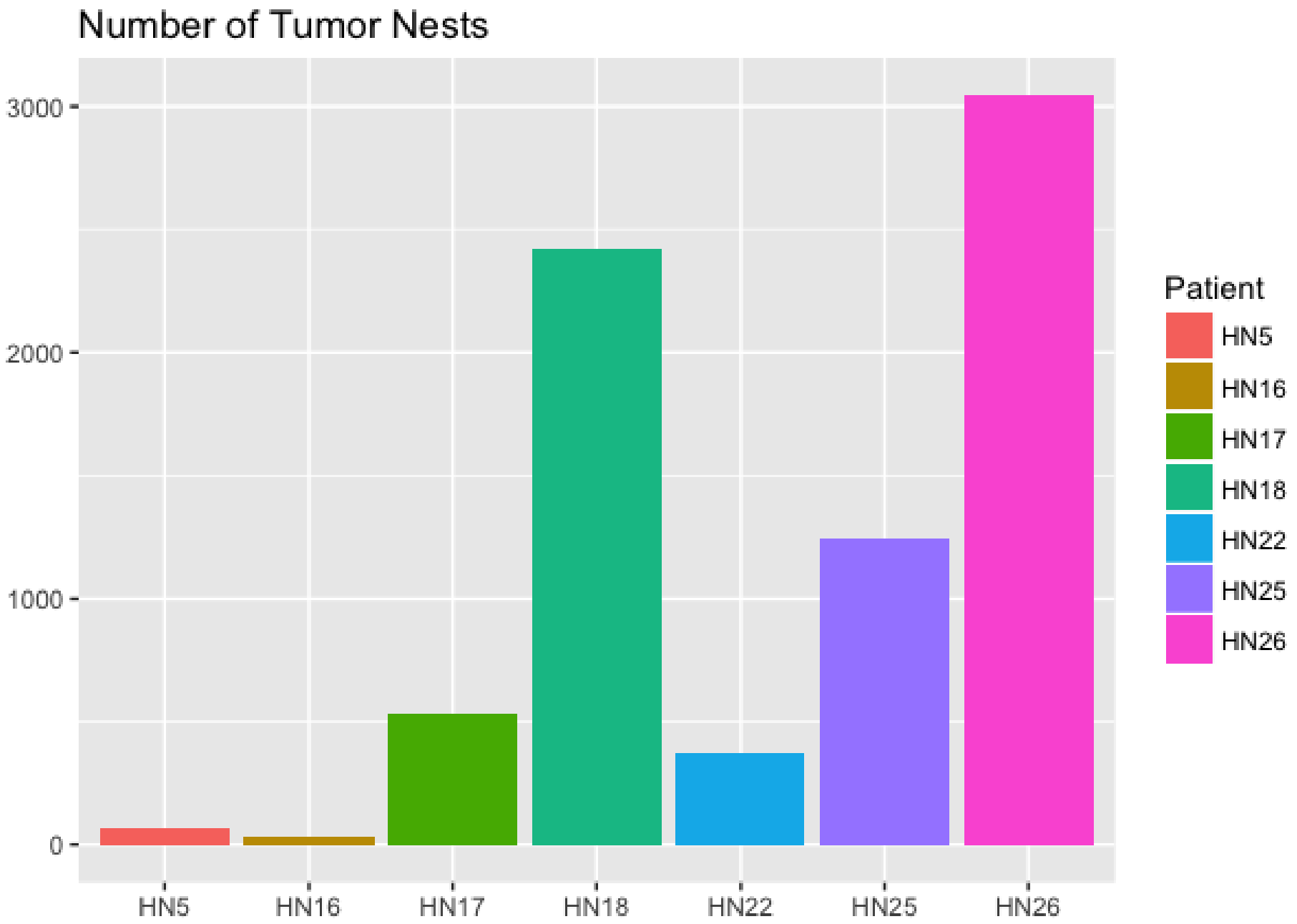
# MEEI 17

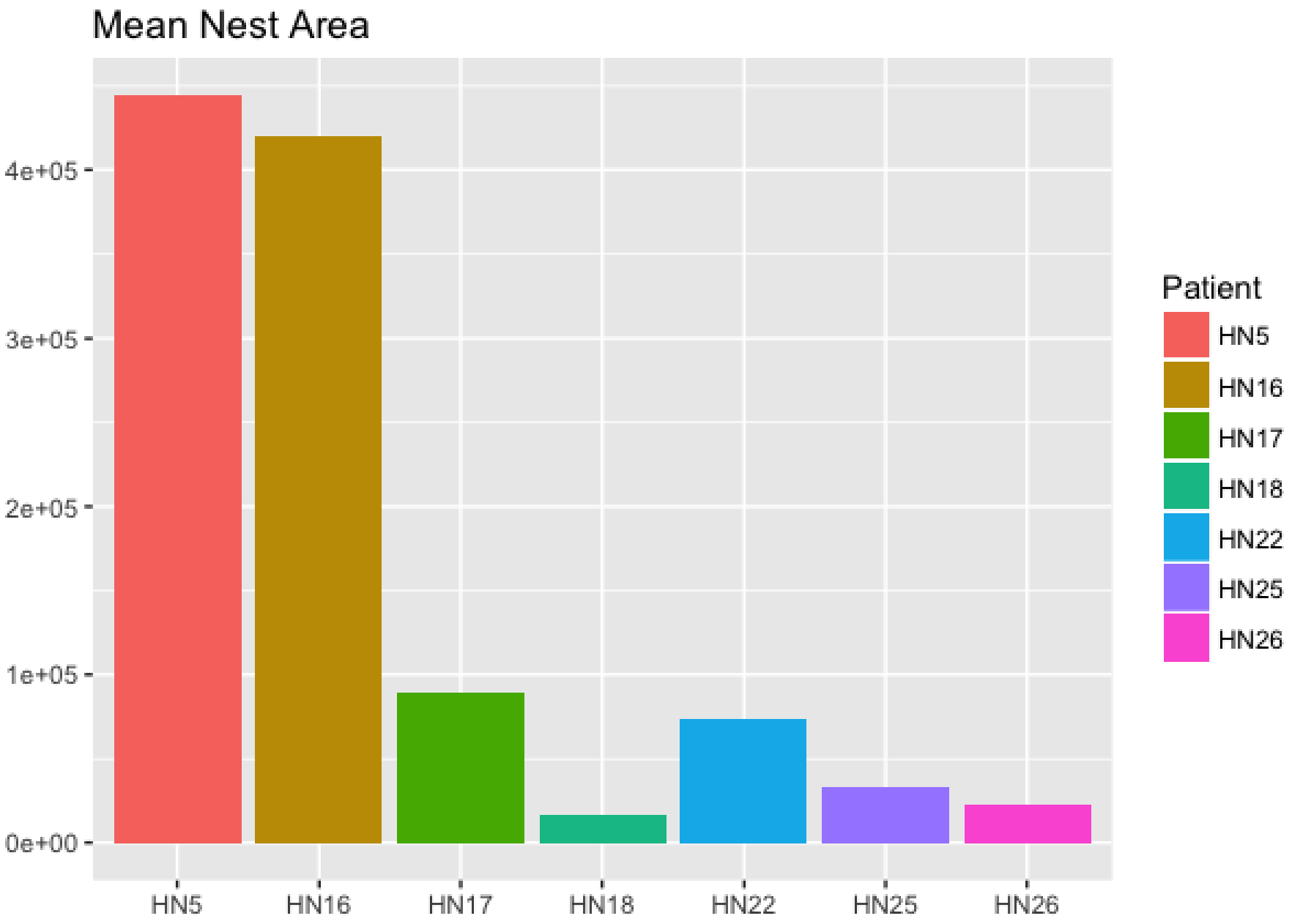

VIM
9619
PDPN
6319
LAMC2
750
LAMB3
8180
SPRR1B
100885
15376
967
117
397
7288
79
505
223
3221
2537
8544
186
11120
50485
15160
7523

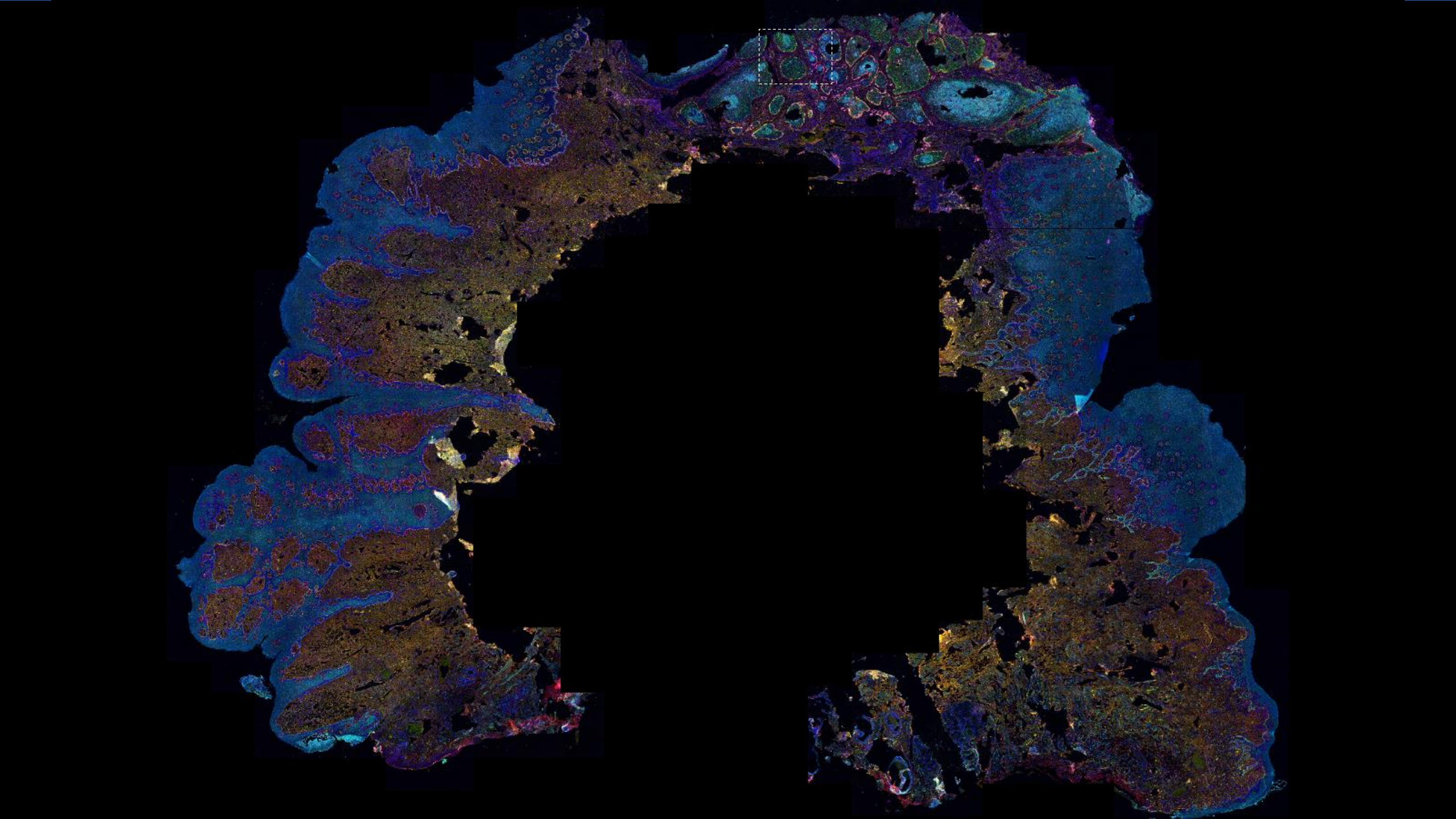

VIM
PDPN
LAMB3
LAMC2
SPRR1B
VIM
PDPN
LAMB3
LAMC2
SPRR1B
VIM
LAMB3
LAMC2
PDPN
SPRR1B
Number of Cells in Tumor (+)
SPRR3
VIM
PDPN, LAMB3, LAMC2
Differentiated
Less-differentiated
(Epithelial)
(Mesenchymal)

# Tumor Nest Definition

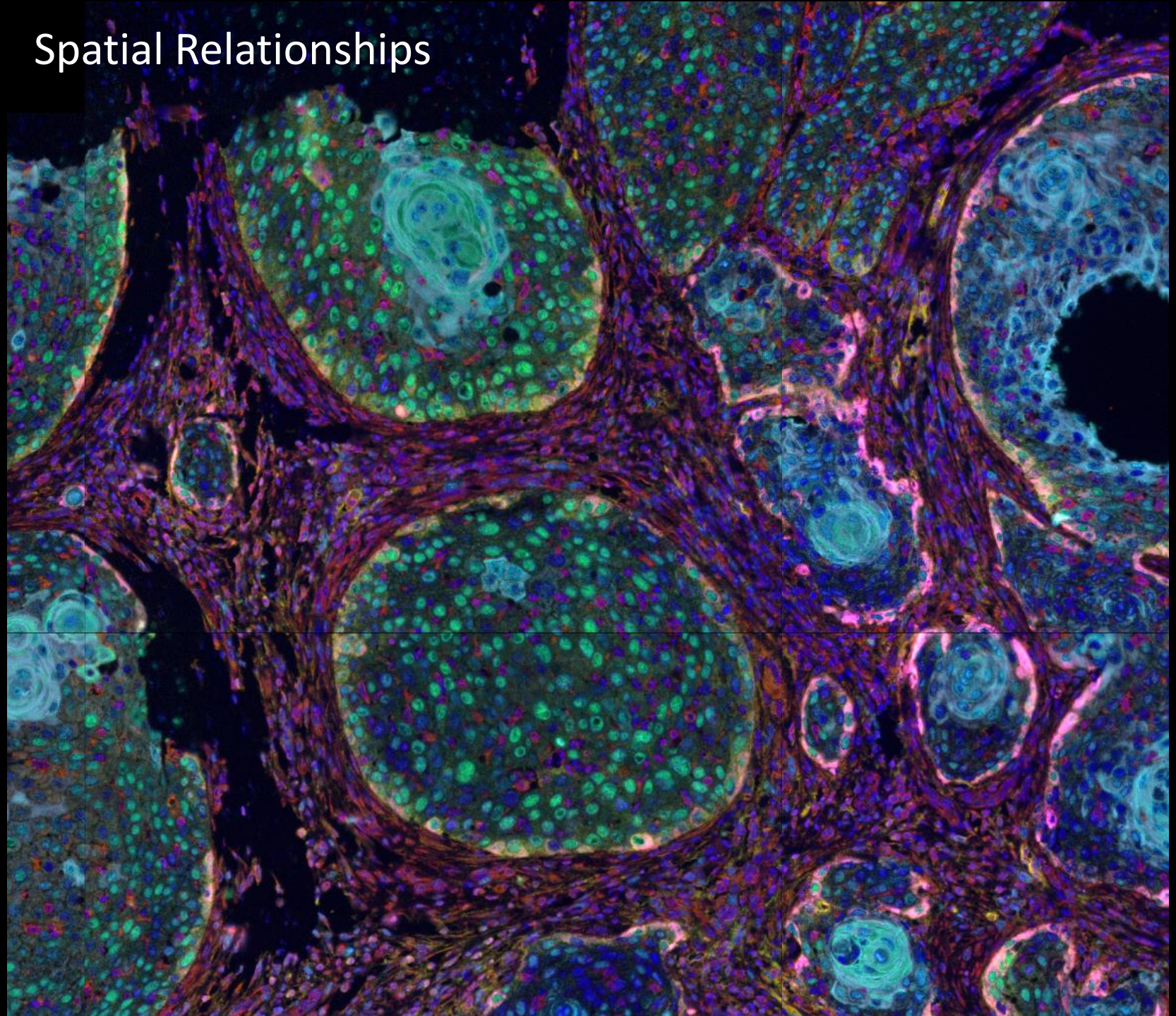



Number of Tumor Nests
3000
2000
1000
0
HN5
HN16
HN17
HN18
HN22
HN25
HN26
Patient
HN5
HN16
HN17
HN18
HN22
HN25
HN26

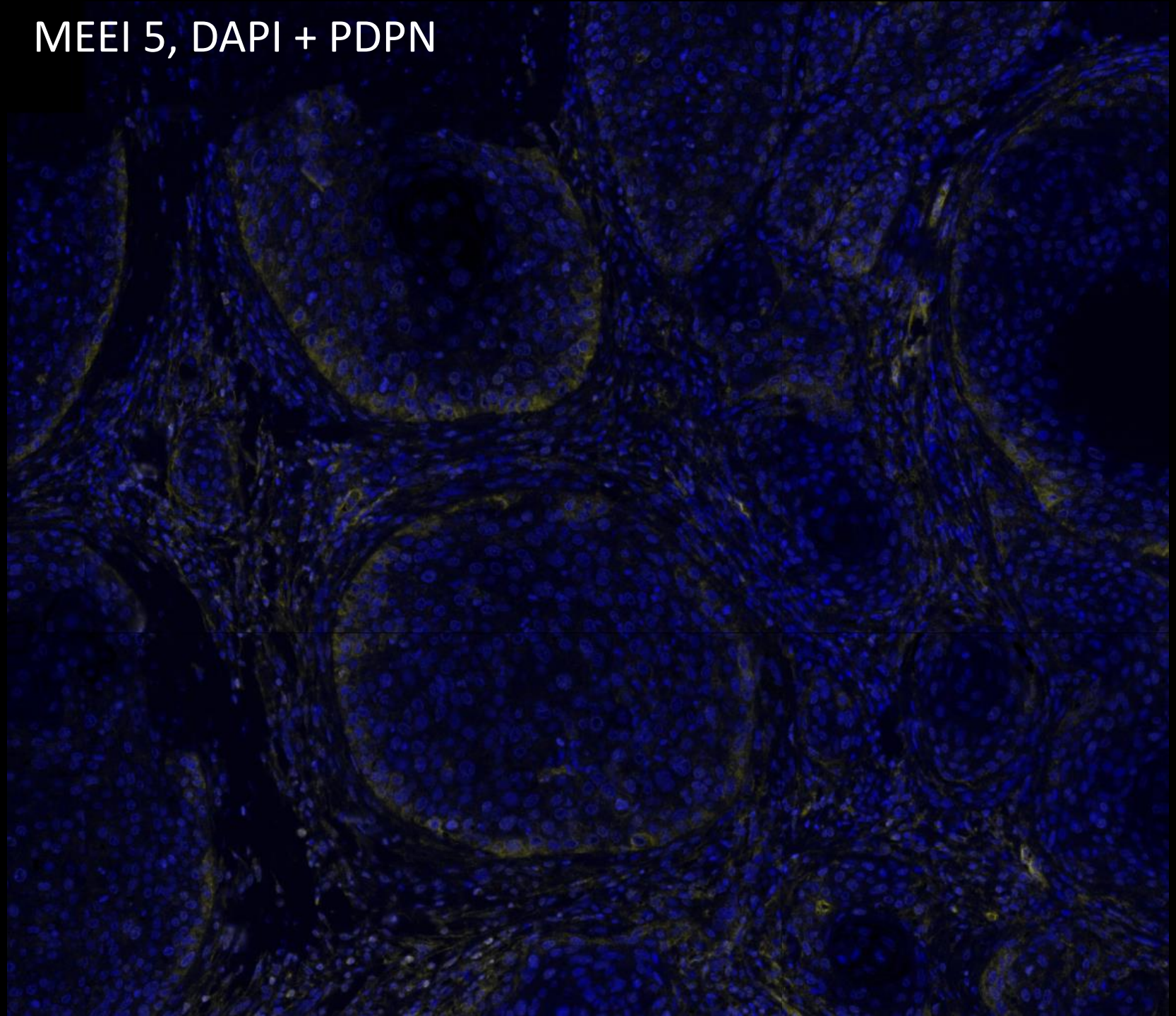

Mean Nest Area
4e+05
3e+05
2e+05
1e+05
0e+00
HN5
HN16
HN17
HN18
HN22
HN25
HN26
Patient
HN5
HN16
HN17
HN18
HN22
HN25
HN26



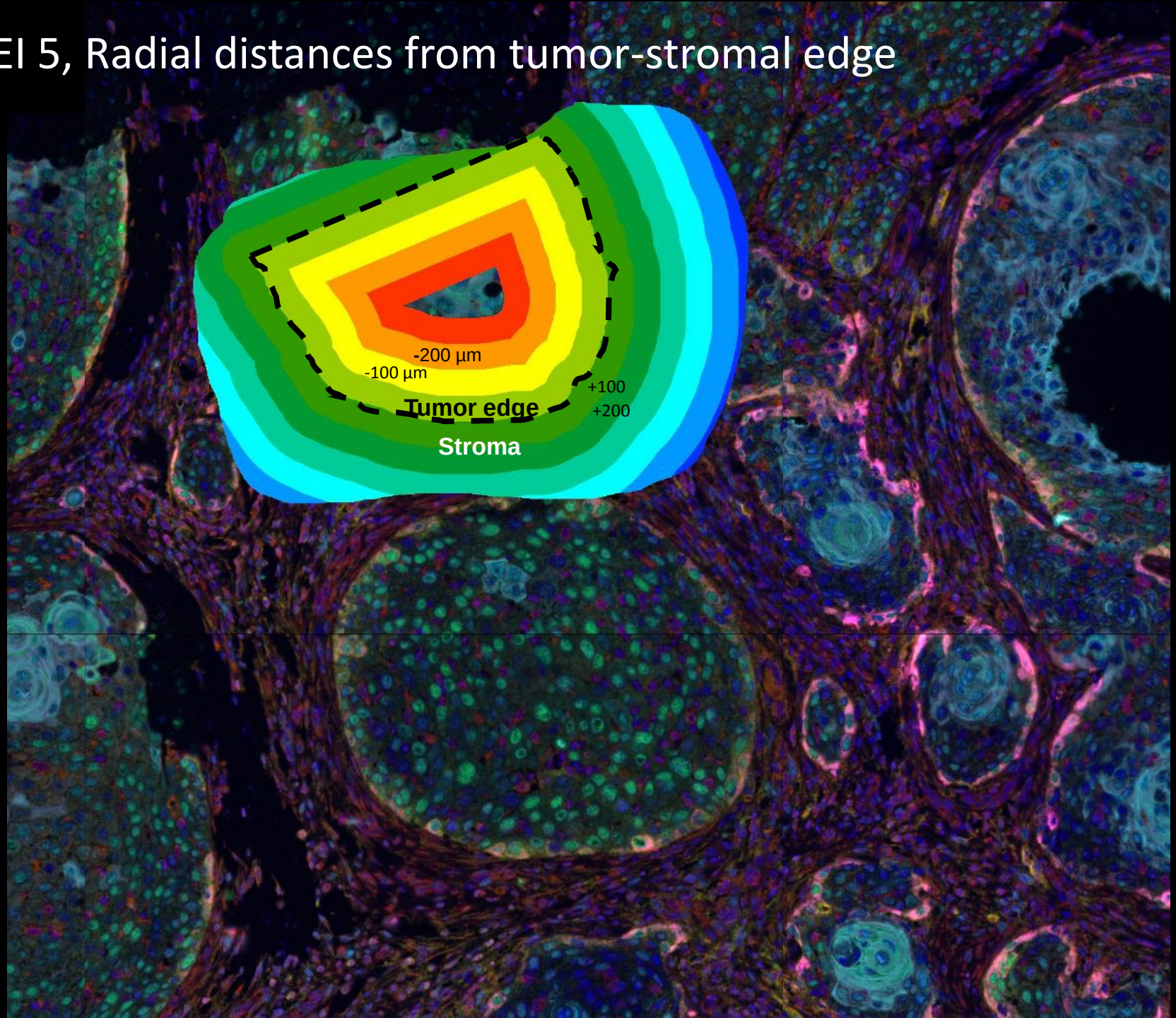
# Spatial Relationships



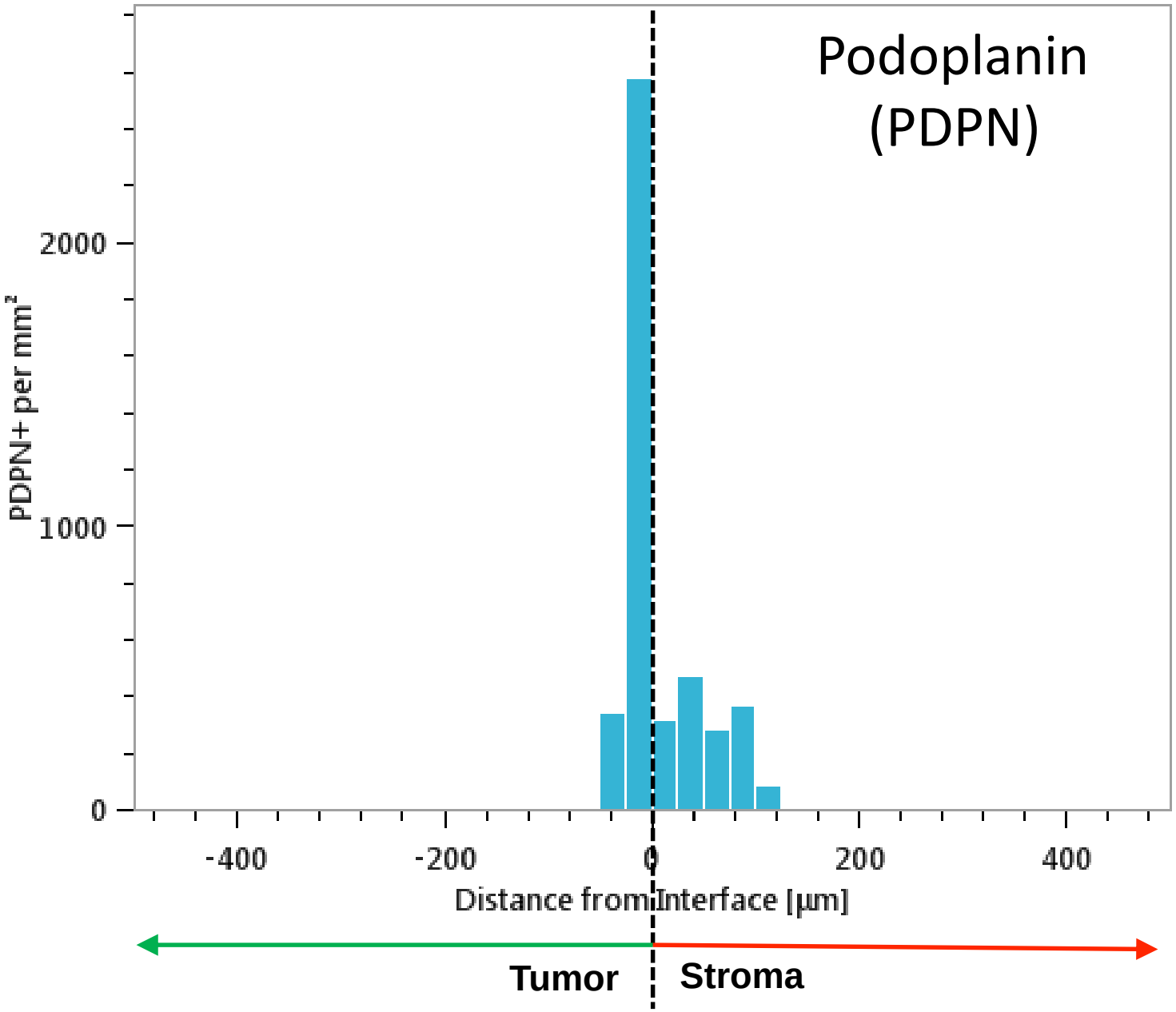
MEEI 5, DAPI + PDPN



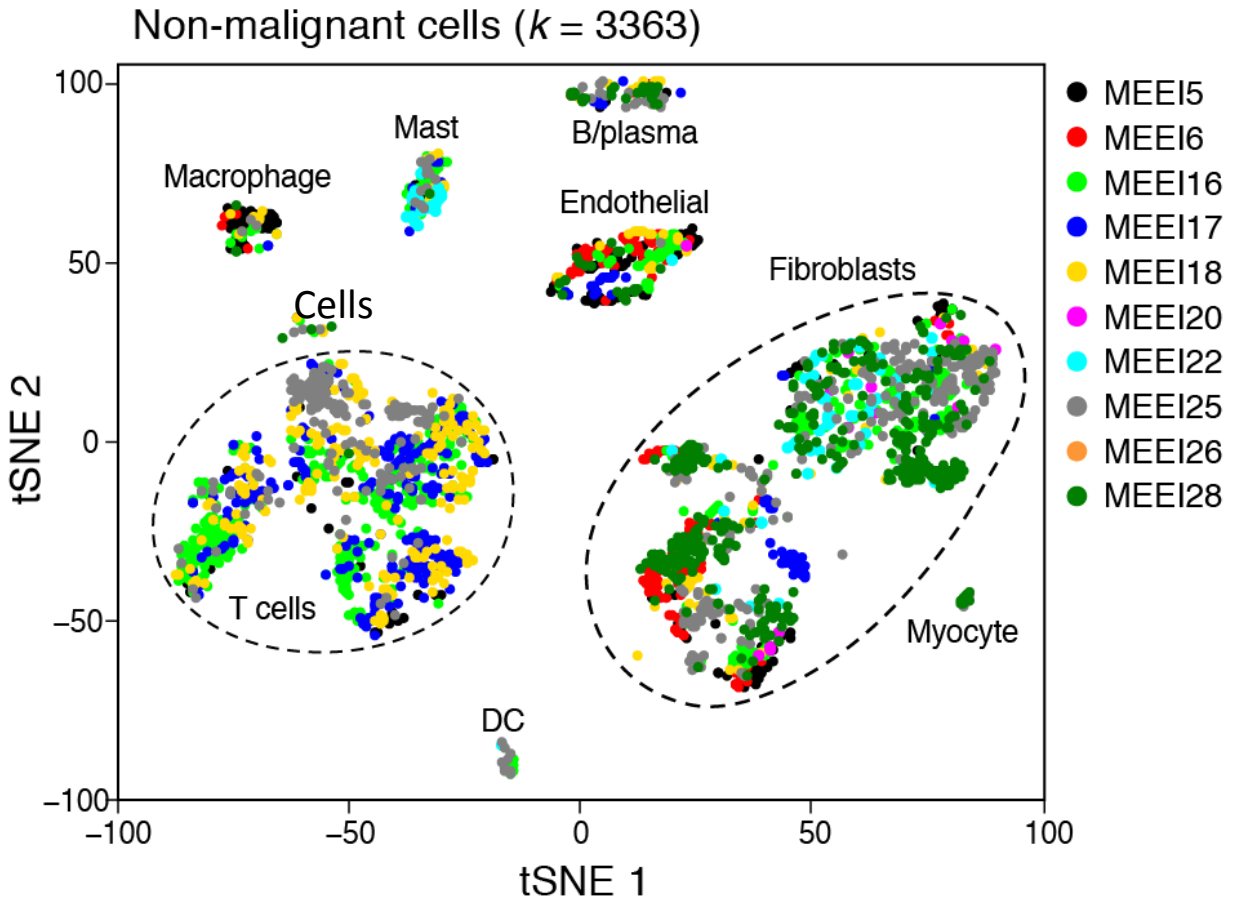
# MEEI 5, Radial distances from tumor-stromal edge

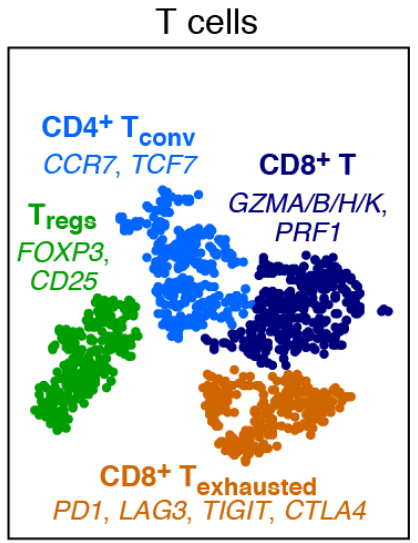


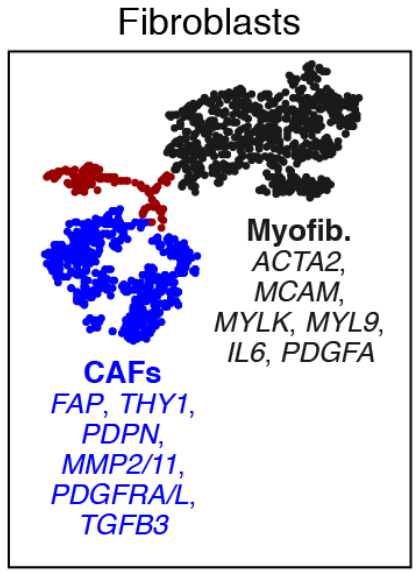
MEEI5


Podoplanin
(PDPN)
PDPN+ per mm²
2000
1000
0
-400
-200
0
200
400
Distance from Interface [µm]
Tumor
Stroma

# Non-malignant Cells



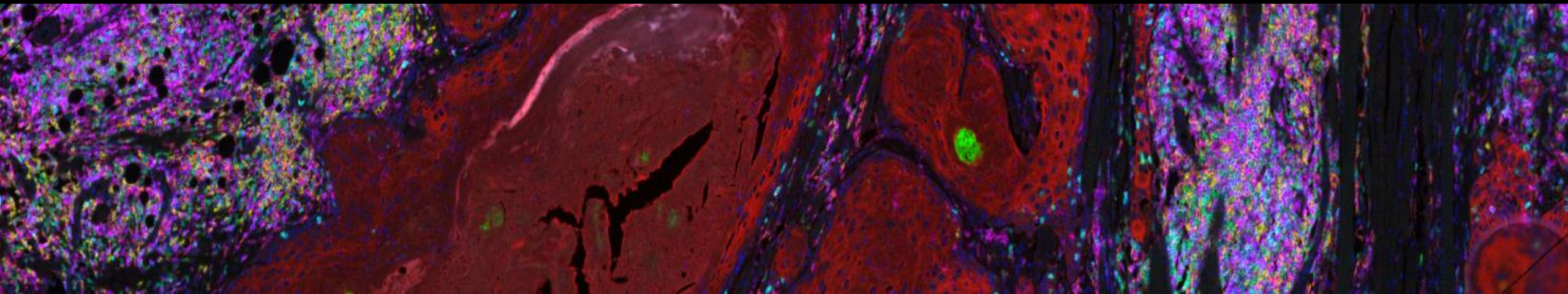



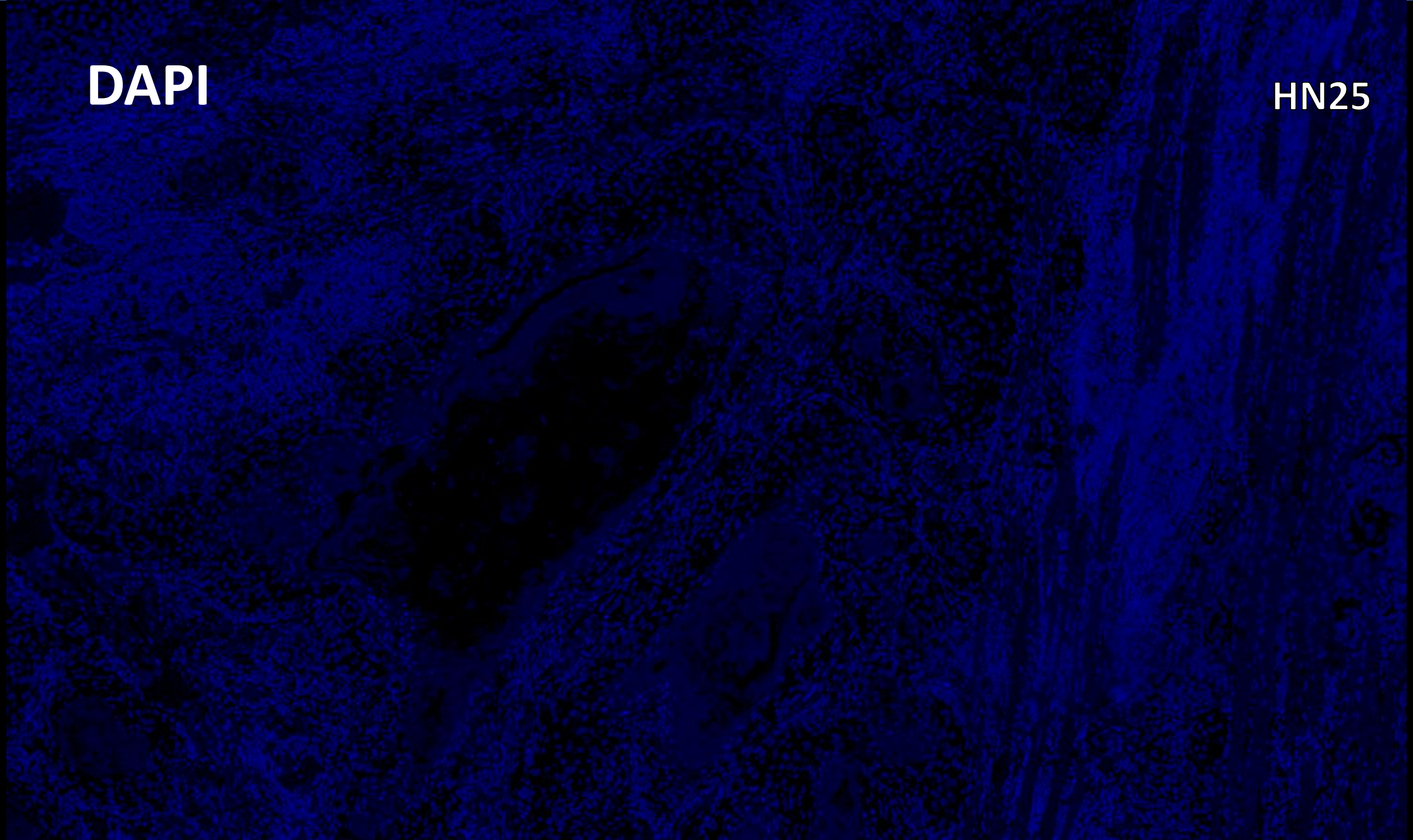




Puram*, Tirosh*, Parikh* et al, *Cell* (2017)

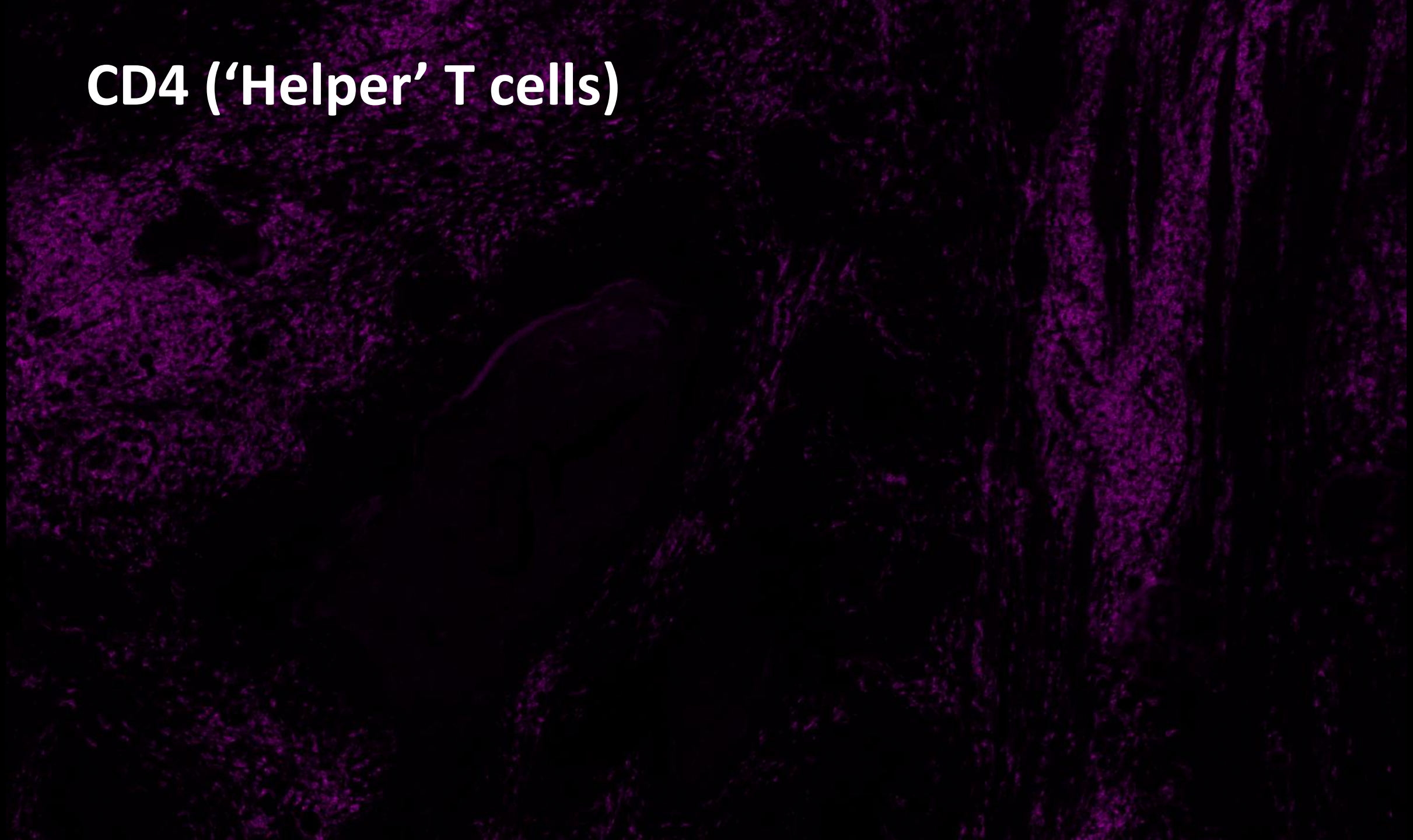
# Head and Neck MSI Tissue Staining

| Exhaustion | Immune | Tumor |
| --- | --- | --- |
| PD-1 | CD4 | CK |
| | CD163 | |
| | CD8 | |
| | CD20 | |



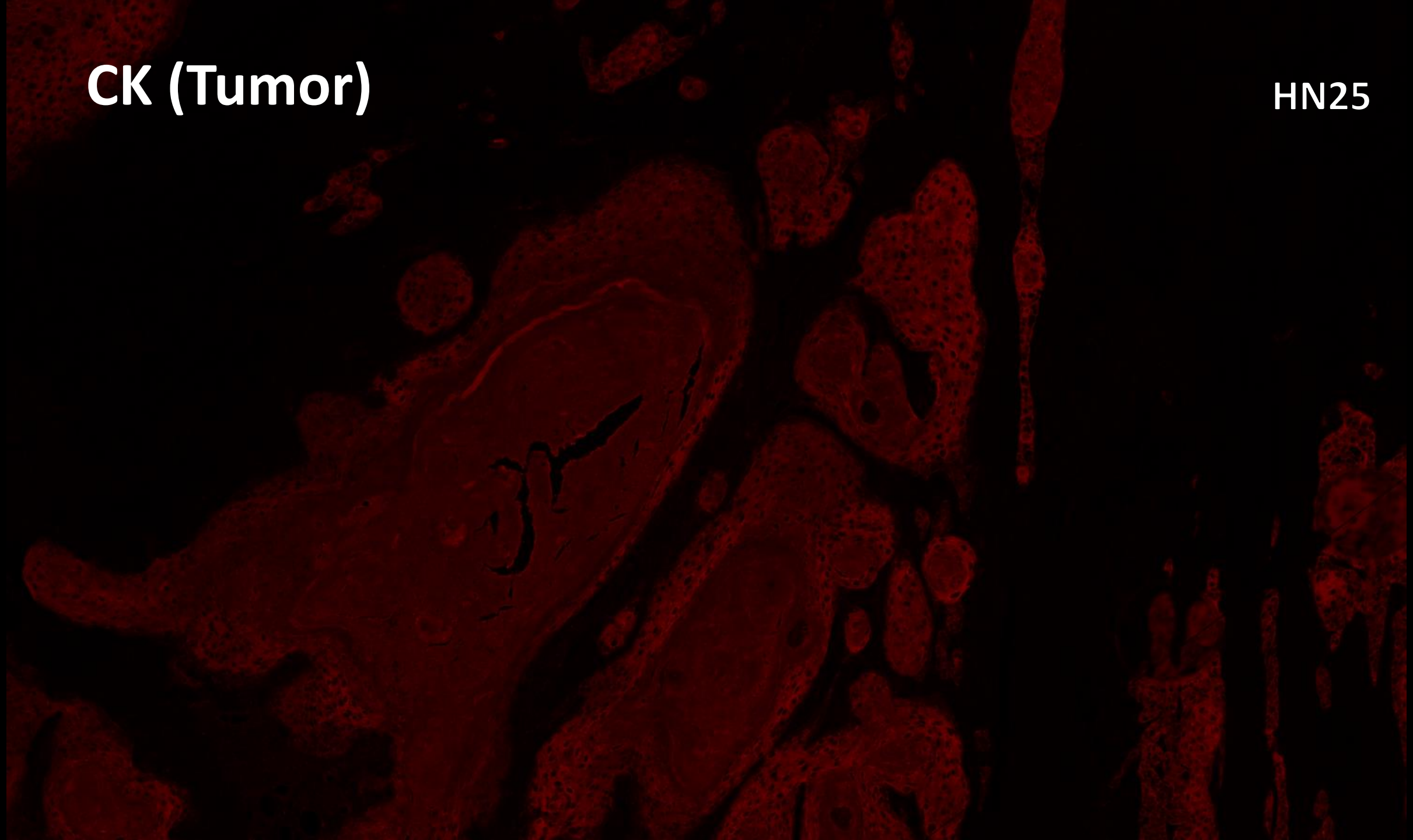

DAPI
HN25

# CD4 (‘Helper’ T cells)



# PD-1 (Exhaustion Marker)

HN25

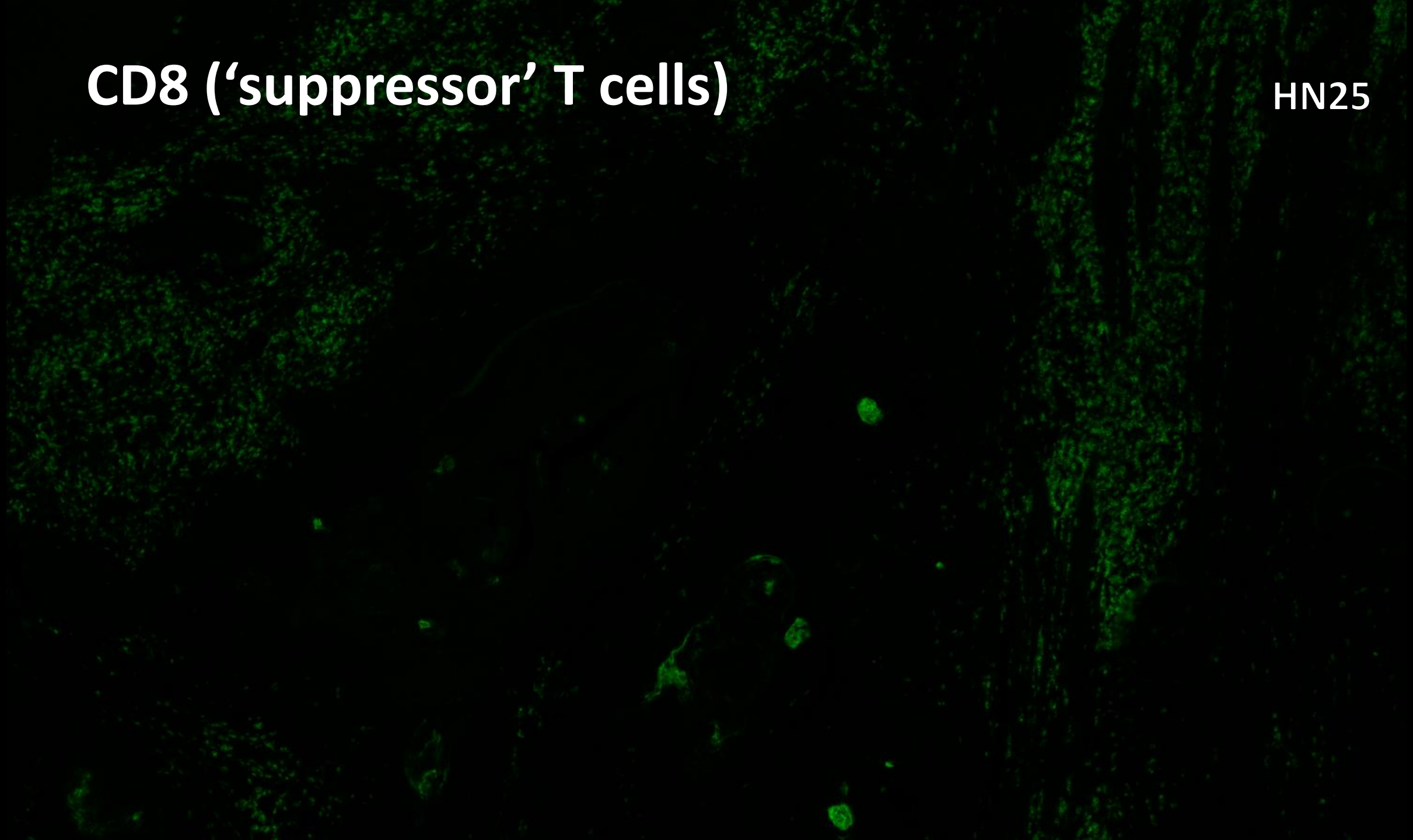

CK (Tumor)
HN25

# CD163 (Macrophage)

HN25

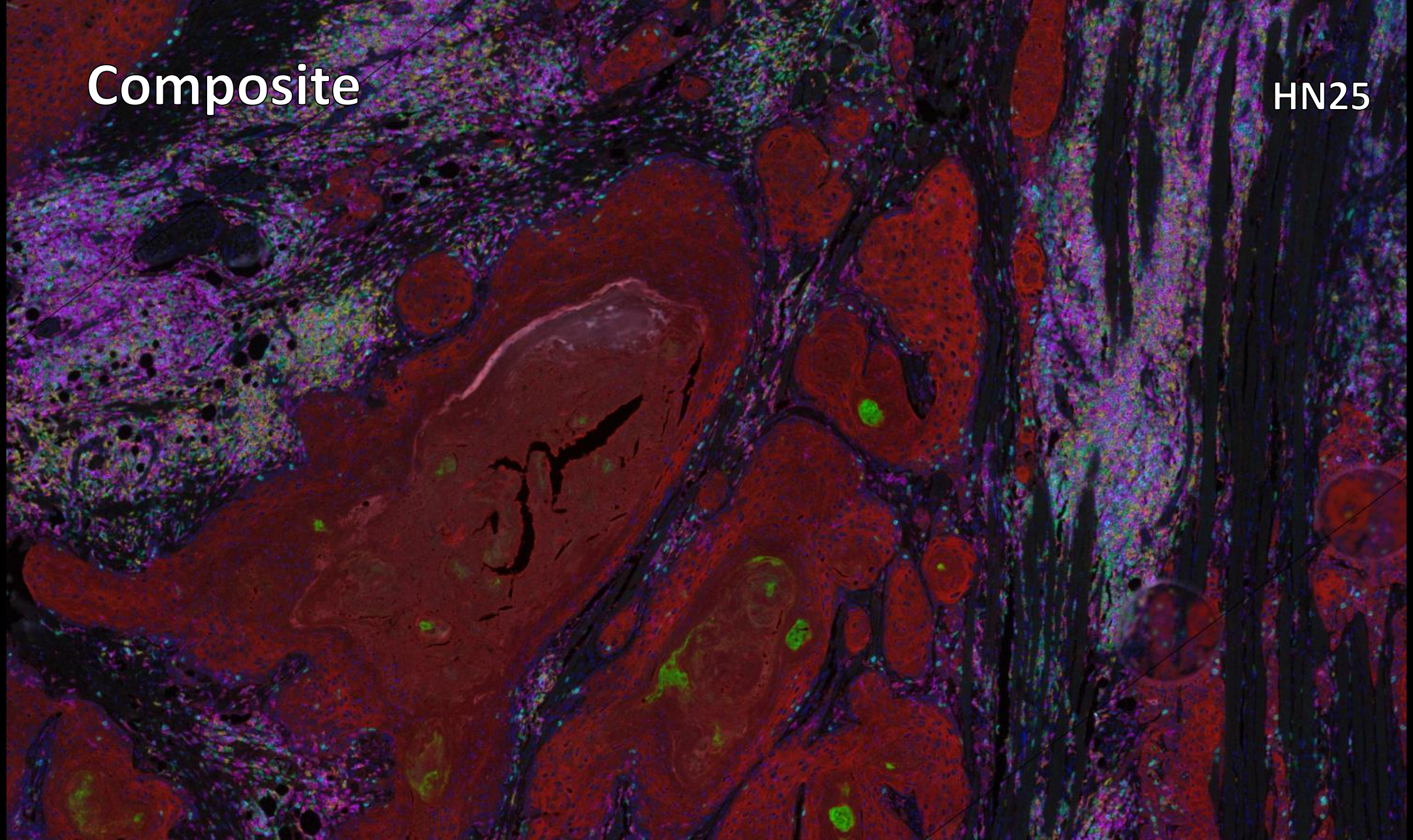
# CD8 (‘suppressor’ T cells)

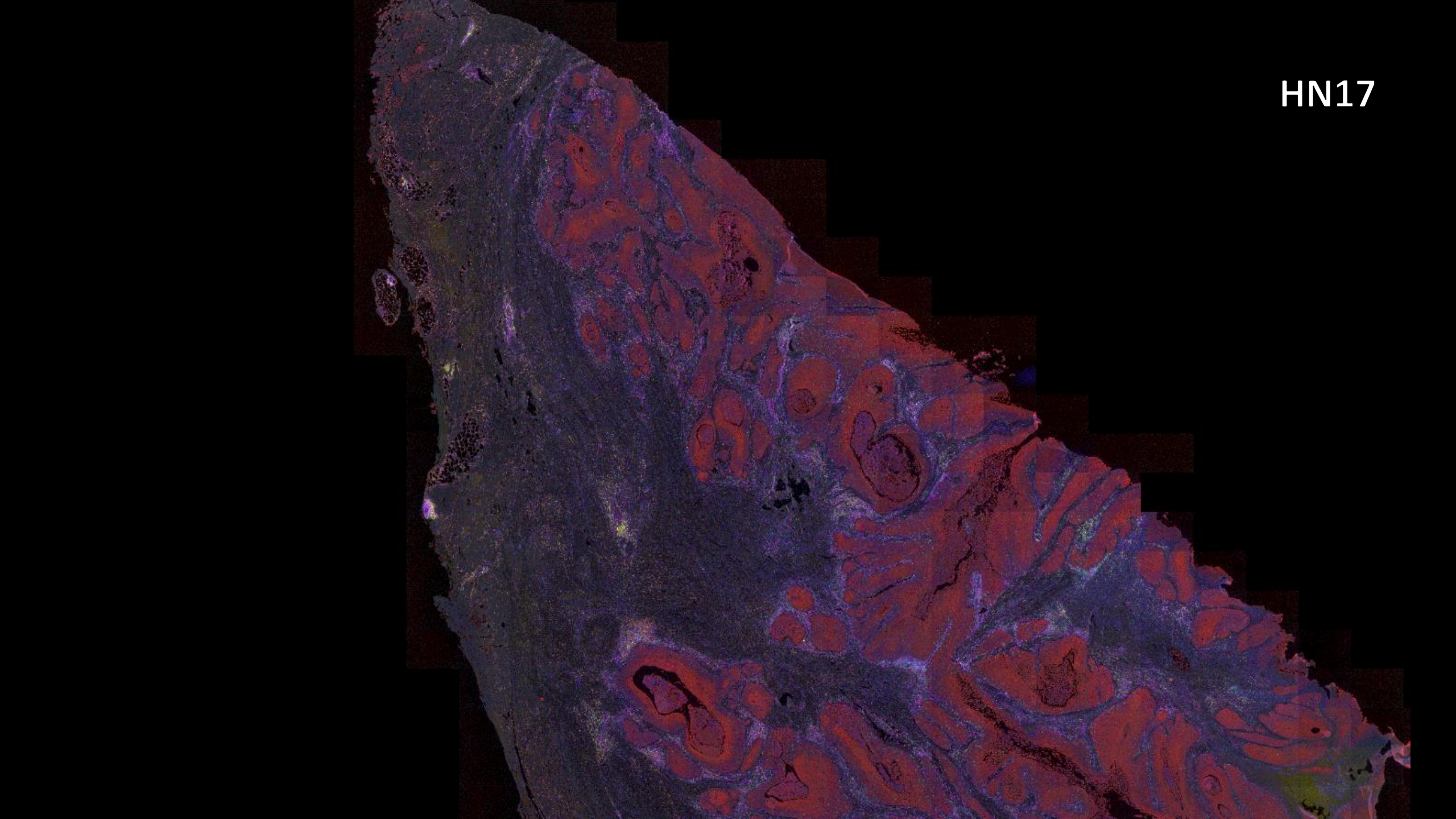
HN25

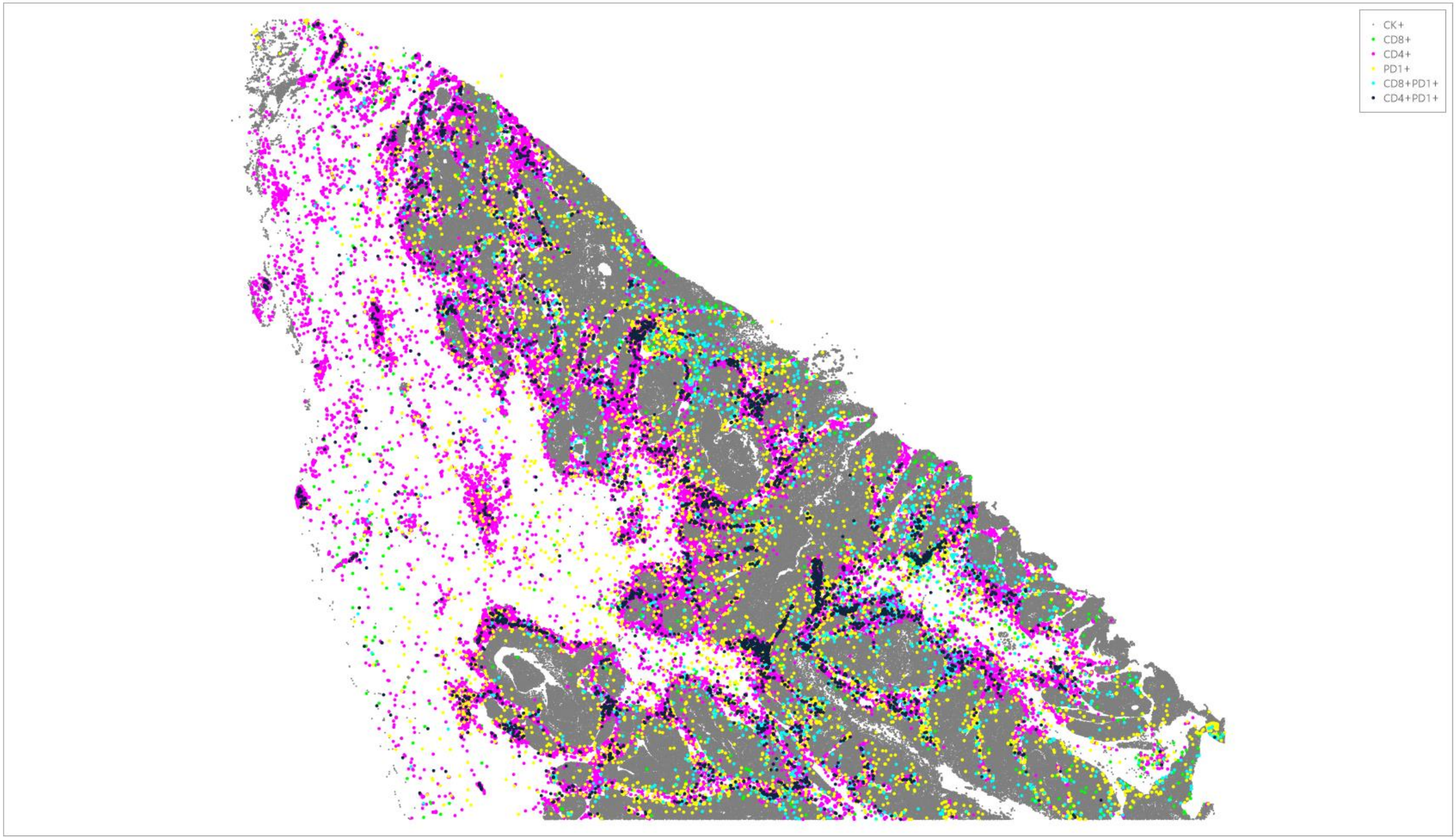


# CD20 (B Cell)

HN25


Composite
HN25


HN17


CK+
CD8+
CD4+
PD1+
CD8+PD1+
CD4+PD1+

# Summary

**MSI**

- 7 color tissue staining per section
- Quantitative signal analysis
- Maintain tumor architecture and spatial relationships
- Automated analysis of 100,000+ cells per tissue

- p-EMT markers (PDPN, LAMC2, LAMB3) co-localize at tumor edge
- Immune panel

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