Spatial transcriptomic analysis of canine metastatic melanoma: Defining RNA signatures of primary tumors and the brain microenvironment

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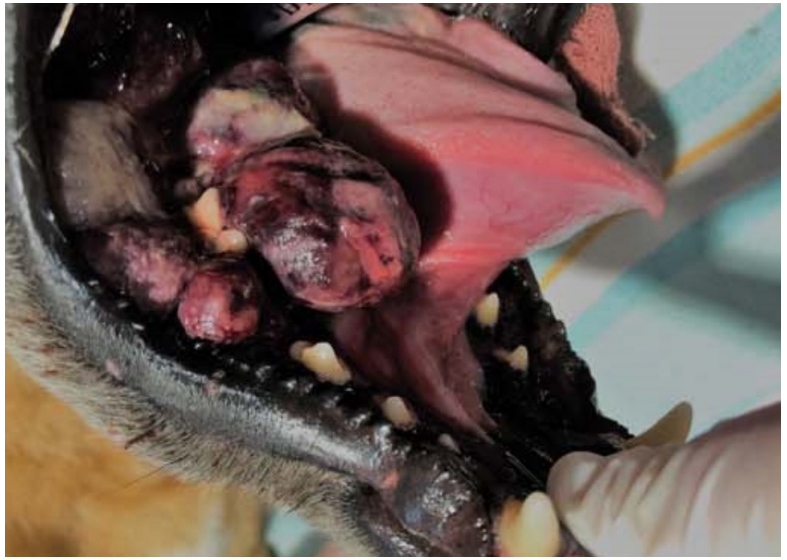
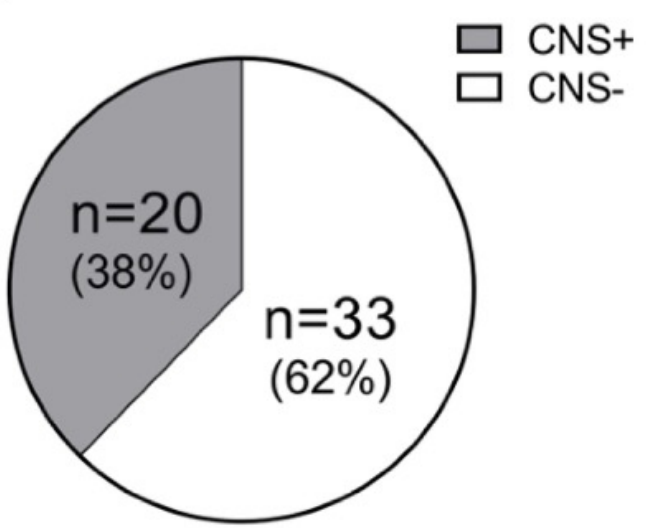
STAR 2023 Oral Presentation

Features of Canine Malignant Melanoma

- Malignant melanoma (MM) accounts for 7% of all canine cancers
- Oral cavity is the most common primary tumor site – also the most deadly

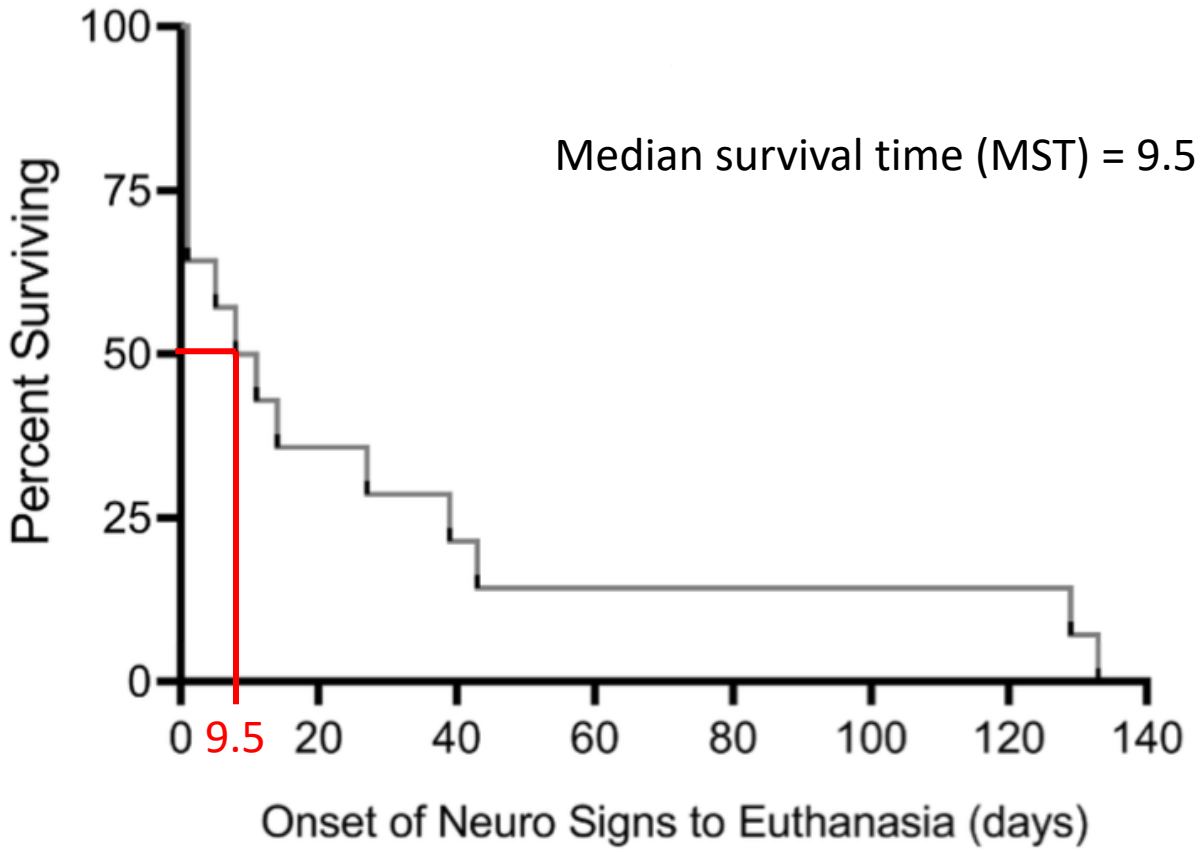


- 38% of MM will have central nervous system (CNS) metastasis



Simpson et al., *Pigment Cell Melanoma Research*, 2014
Razmara et al., *Frontiers in Oncology*, 2022

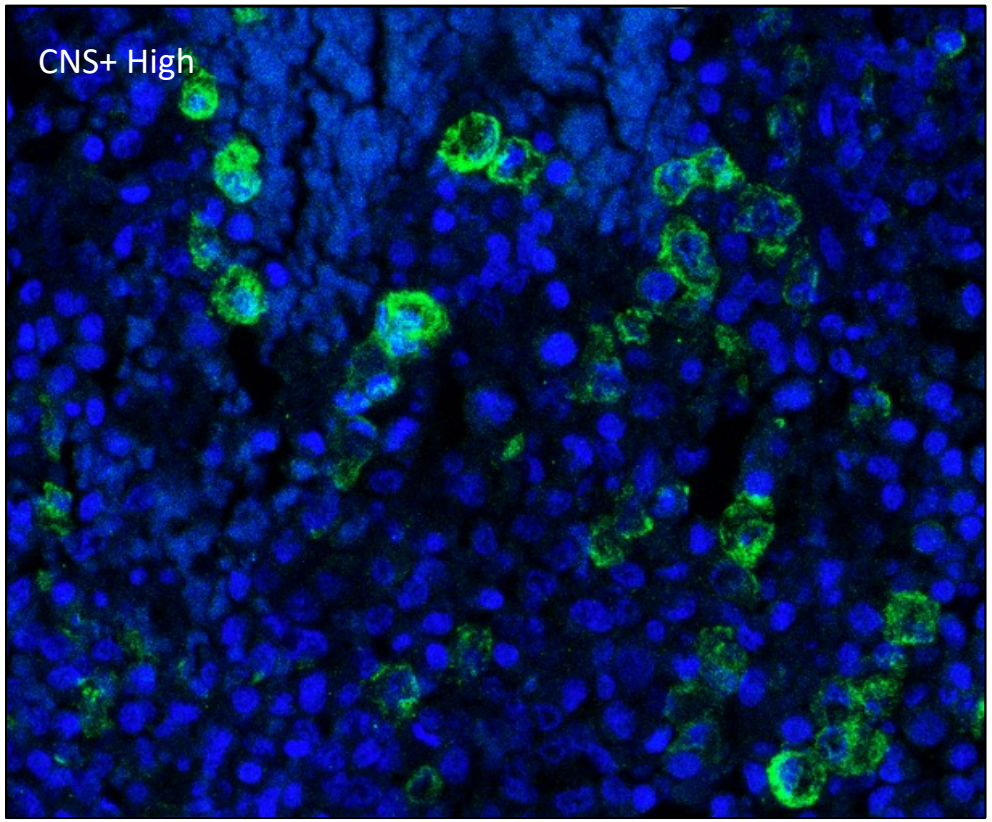
Presence of clinical brain disease accelerates euthanasia

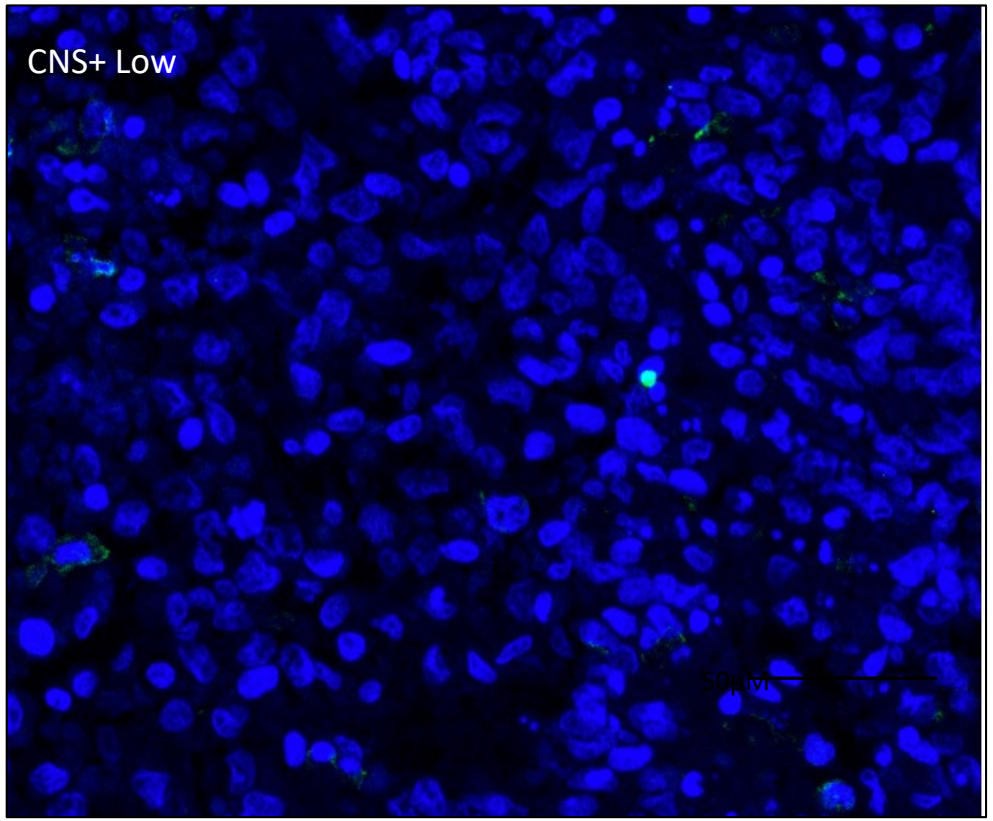


Median survival time (MST) = 9.5 days

Razmara et al., *Frontiers in Oncology*, 2022

Microglia have been implicated as being permissive to brain metastasis





Consales et al., unpublished observations, STAR 2021

Hypothesis: *The brain microenvironment and primary tumor transcriptomic signatures will be distinct between groups, correlating with the presence or absence of brain metastasis in canine metastatic melanoma.*

- Aim #1: Identify the transcriptomic differences of cells within the brain microenvironment in the following canine groups: Malignant melanoma brain 1) with and 2) without brain metastasis, and 3) Normal brain.
- Aim #2: Identify transcriptomic differences of the primary tumor microenvironment in canine malignant melanoma between dogs with and without brain metastasis.

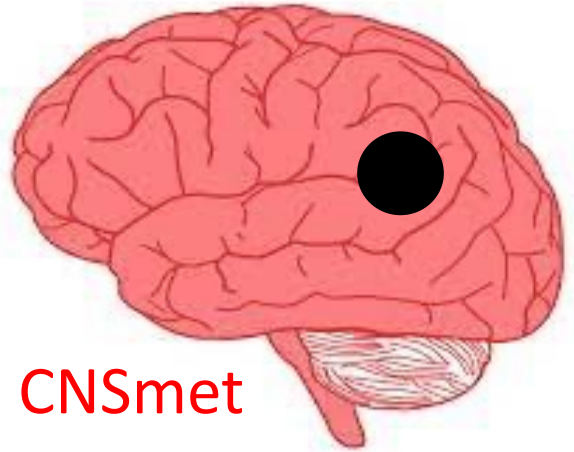
Study Design: Case Selection

Inclusion criteria:

- Histopathological diagnosis from a board-certified pathologist of oral melanoma that has metastasized to at least one organ
- Brain histopathological evaluation
- Tissue availability of brain and primary oral melanoma tumor

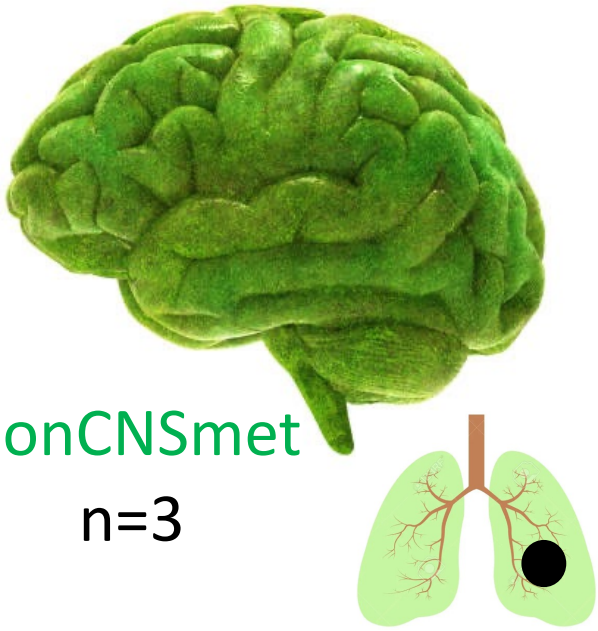
Exclusion criteria:

- Coexisting metastatic cancer
- Additional brain disease



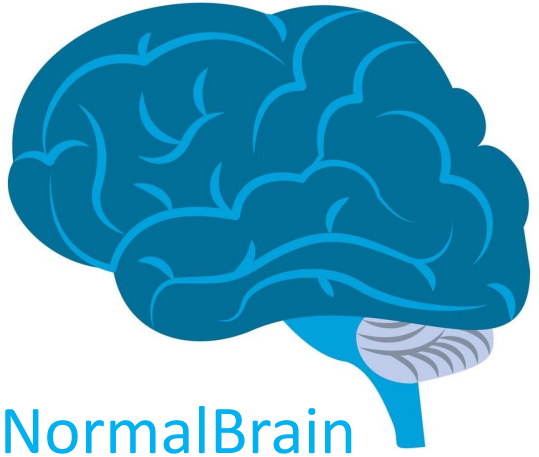
CNSmet

n=3



NonCNSmet

n=3

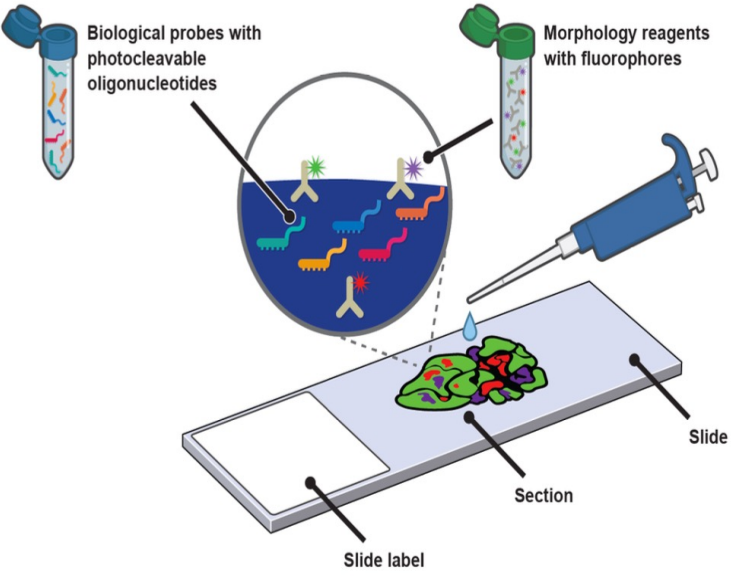


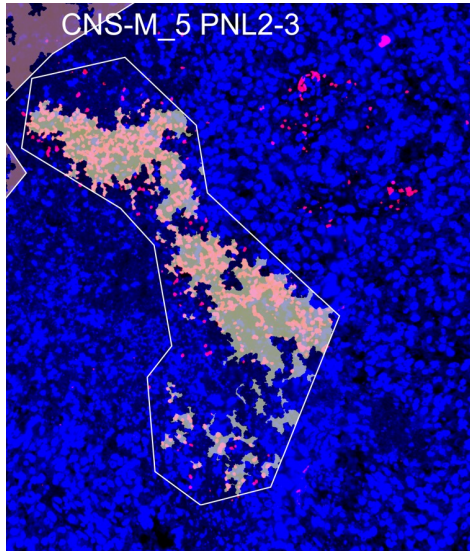
NormalBrain

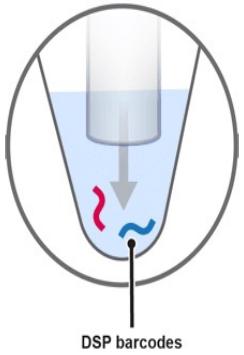
n=3

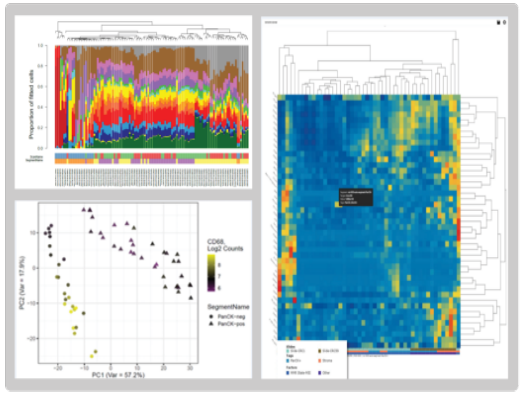
Study Design: Spatial Transcriptomics

Sample Prep → Image and Profile → Count → Analyze









GeoMx Canine Cancer Atlas Panel (1,963 genes)

Microglia = IBA1
Endothelial cells = CD31
Melanocytes = PNL2

Image adapted from Nanostring Website

Data: Quality Control, Filter, Normalization

Segment QC
- Raw reads < 1000
  - Sequencing failed (ex. pipetting error during library prep)
- Percent aligned reads < 80%
  - Contaminated or low quality
- Sequencing saturation < 50%
  - Depth of sequencing not sufficient to capture low expressing unique targets
- *Negative probe count < 10
  - Background noise could not be estimated
- No template control count > 1000
  - Contamination during PCR
- Minimum nuclei count < 50
- *Minimum surface area < 16,000 um^2

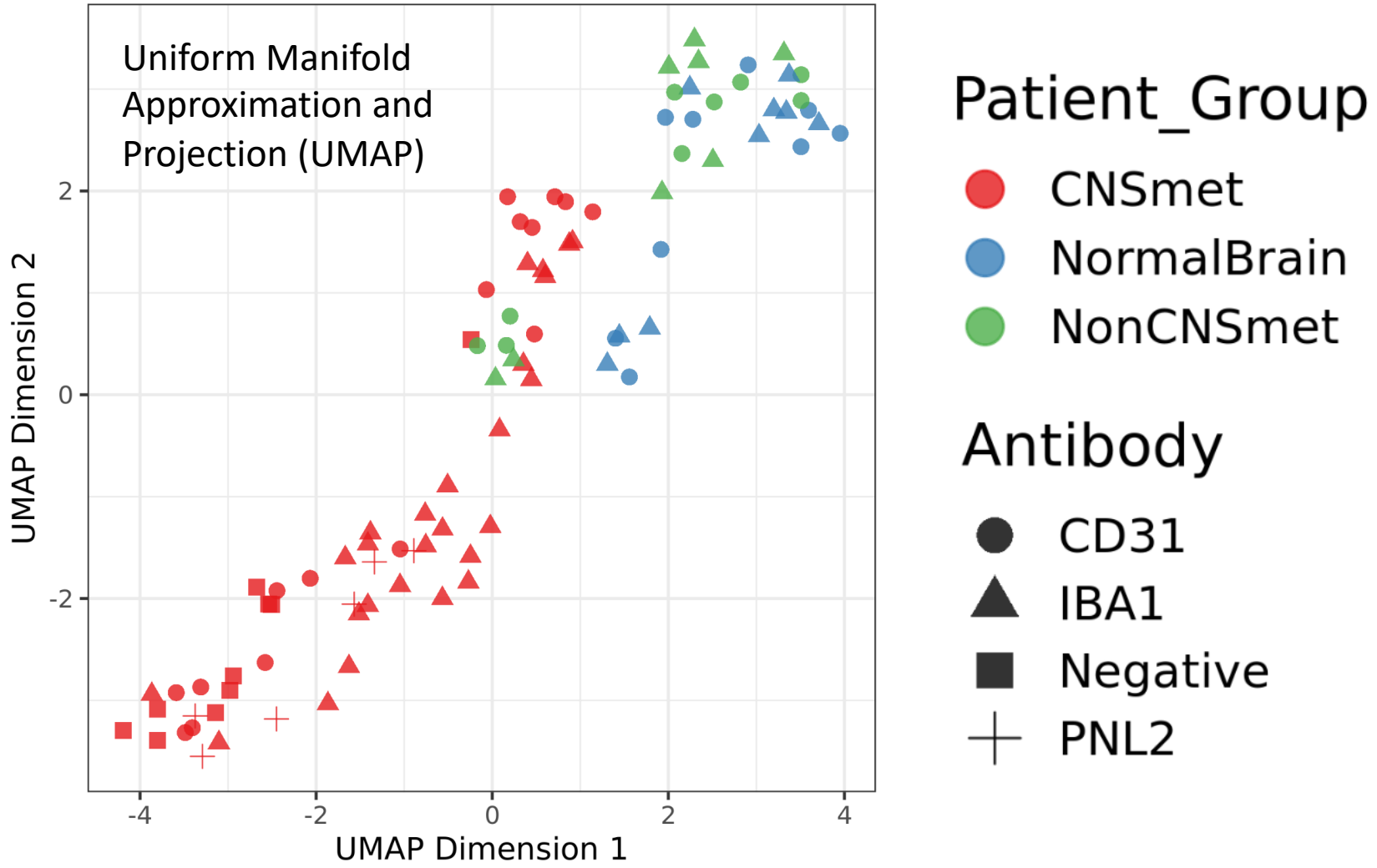
Q3 Normalization

- Normalized to top 25% expressors to reduce variance of gene expression

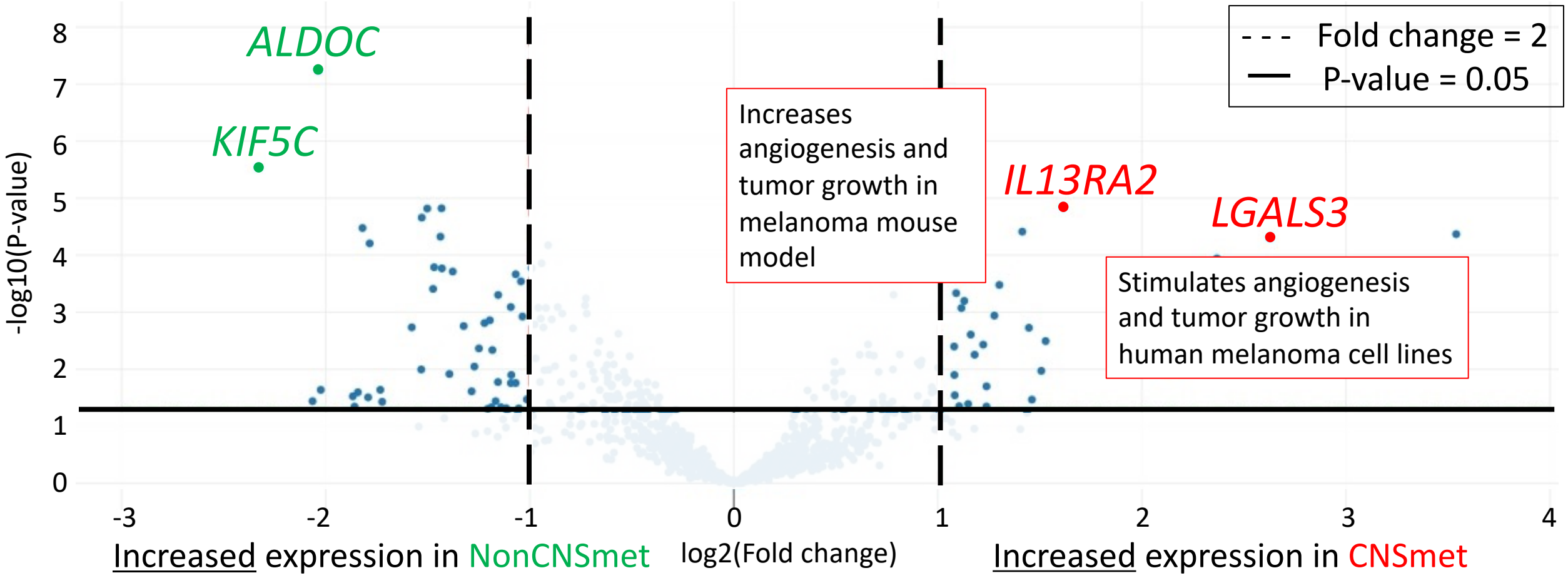
Biological Probe QC
- Probes in all segments/Probes within target < 0.1
  - Excludes probes performing poorly relative to other probes for same target
- Fails Grubbs outlier test in > 20% of segments
  - Excludes probes that are consistent outliers
- Calculate limit of quantitation (LOQ) = 2 SD above mean of negative probes
  - Determines confidence threshold of probe expression
- Results: 2010 total probes = 1997 passed, 13 local outliers

Filter
- By ROI - exclude if expression < LOQ or frequency < 5%
  - Results: 93/100 ROIs passed = 93% of ROIs expressed at least 5% of panel genes
  - Removed all PNL2 ROIs in one case
- By target gene – exclude if expression < LOQ or frequency < 5%
  - Results: 1118/1963 genes passed = 57% of target genes from panel were detected in at least 5% of ROIs

CNSmet samples segregate from NormalBrain and NonCNSmet

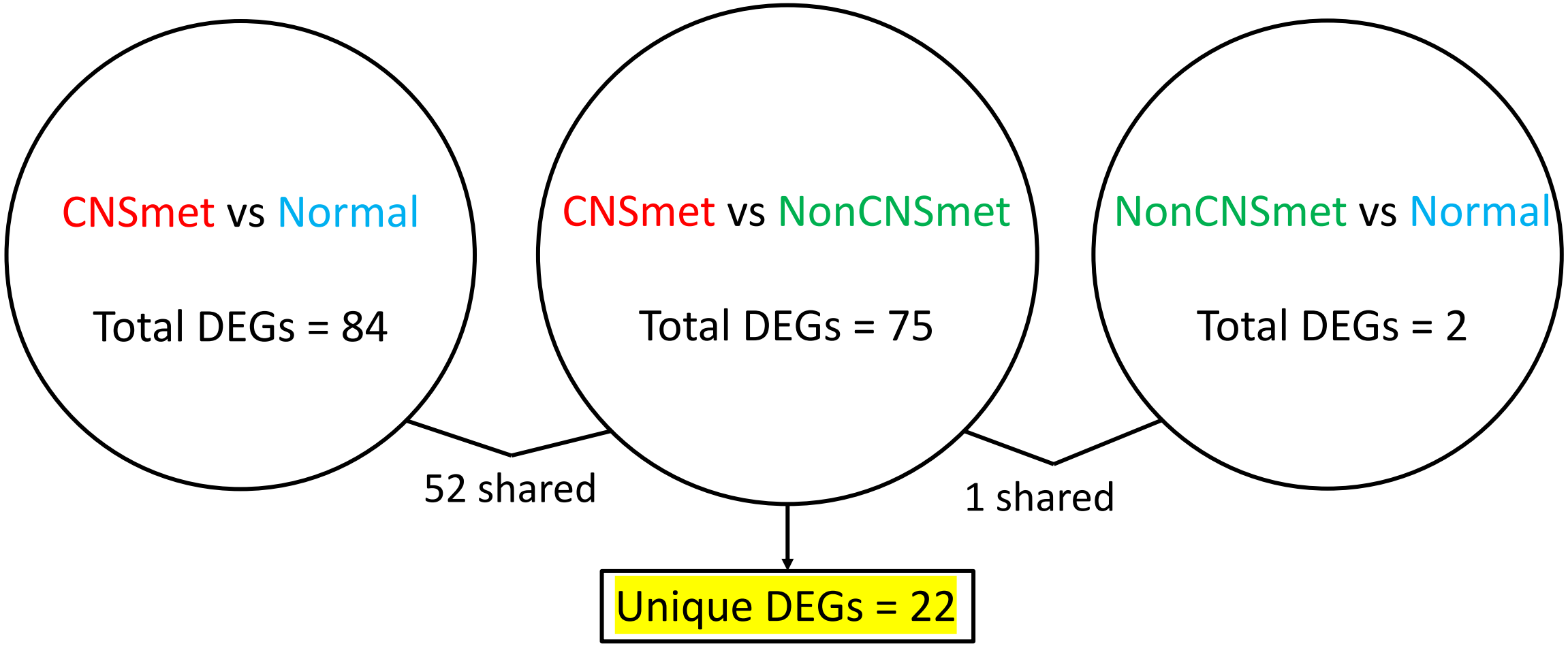


Microglia in dogs with brain metastasis express pro-tumorigenic genes

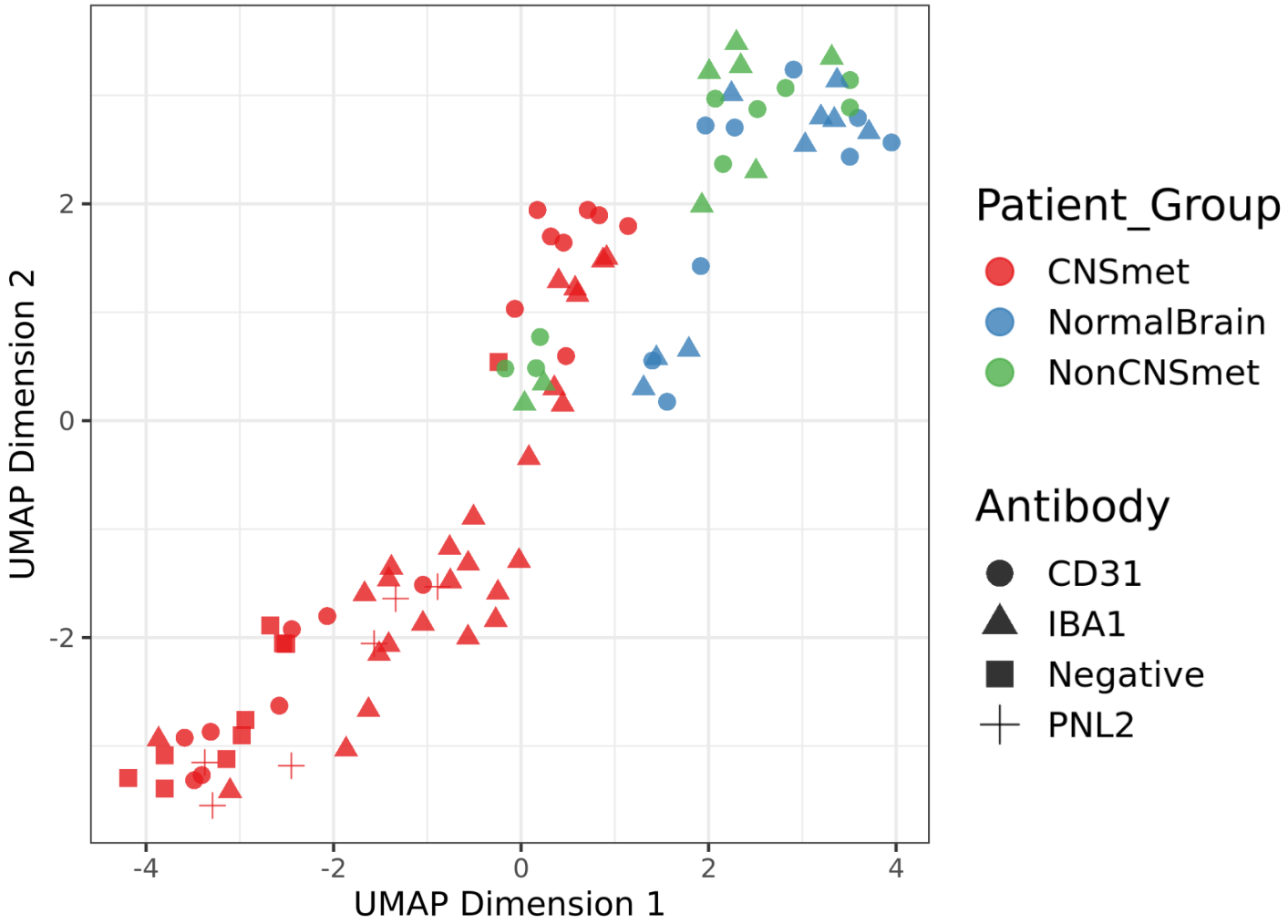


Okamoto et al., *Scientific Reports*, 2019 ; Braeuer et al., *Cancer Research*, 2012

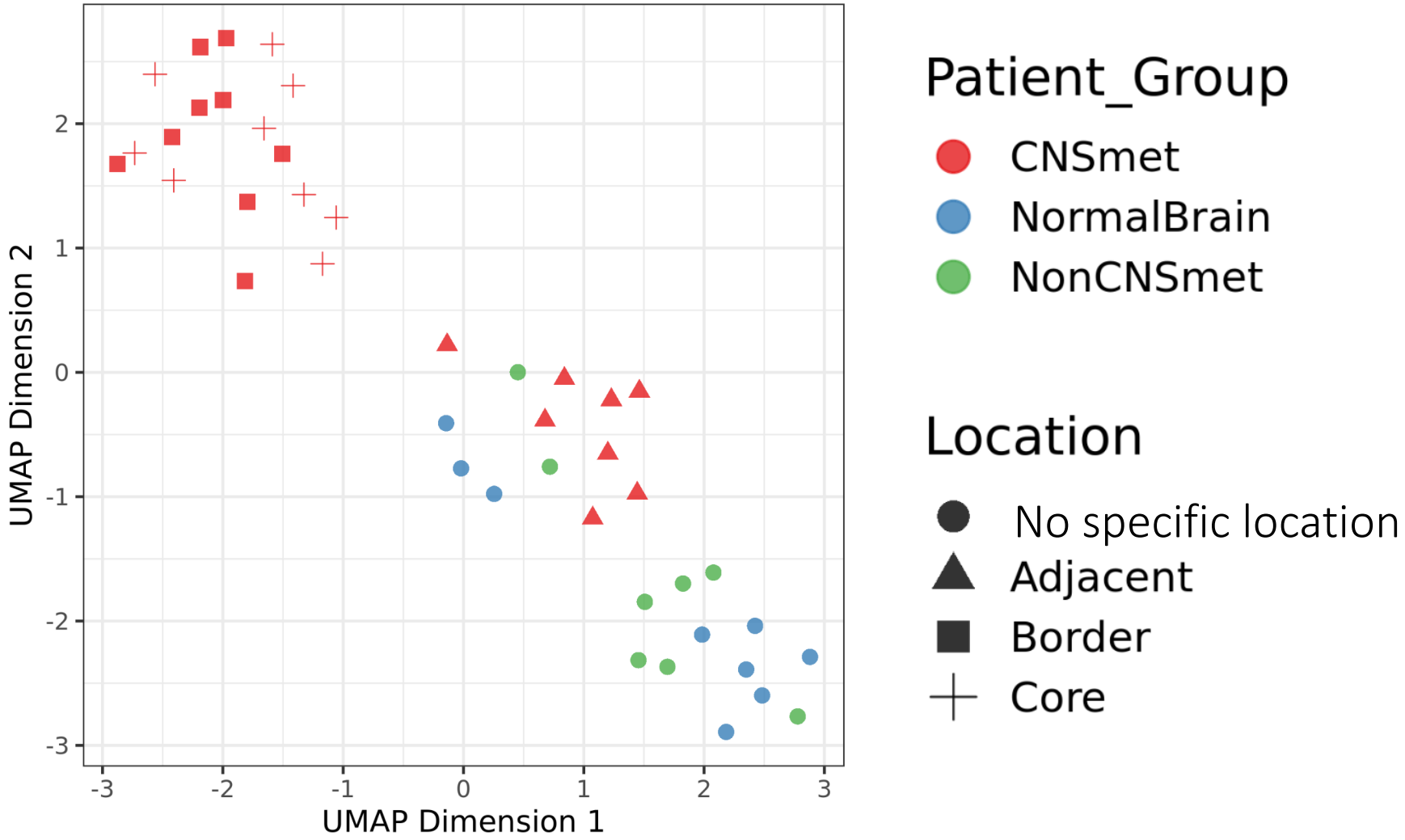
22 unique differentially expressed genes (DEGs) identified in microglia across groups



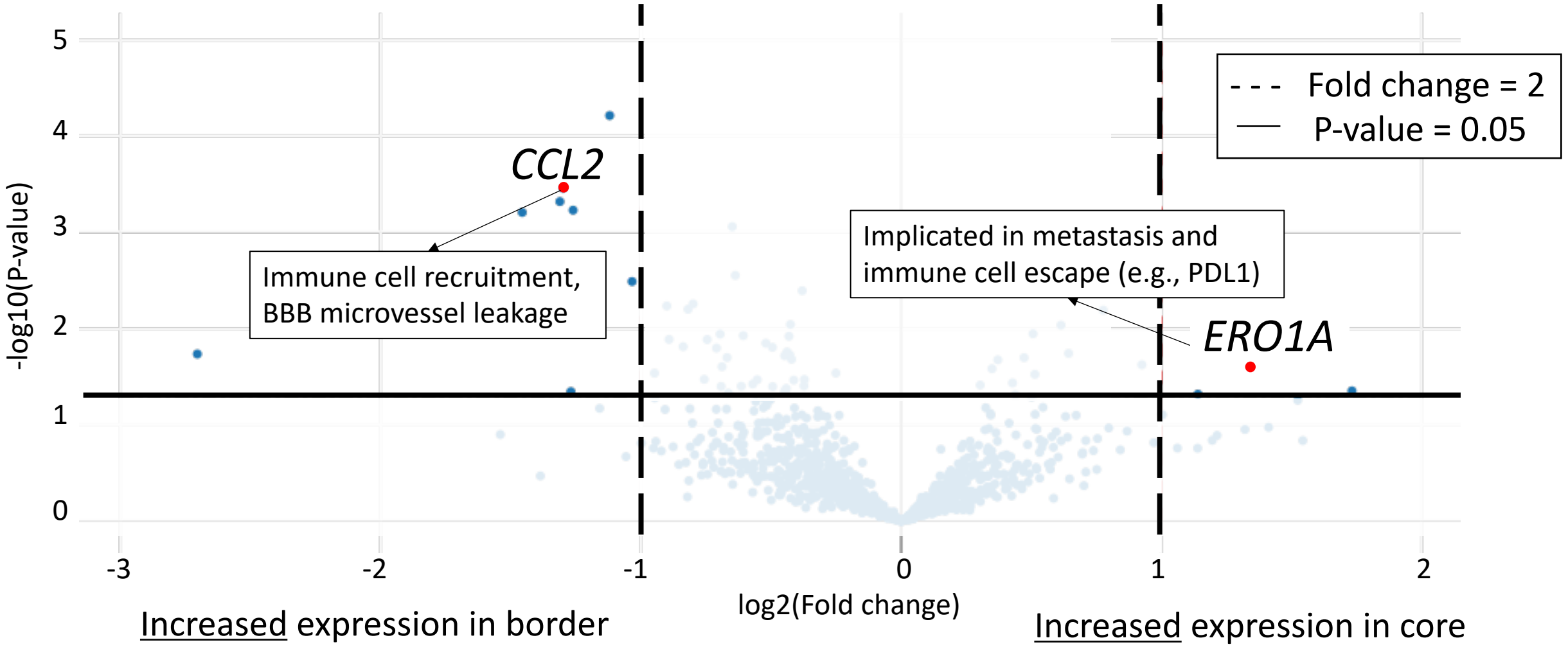
Are there region-specific gene signatures of microglia within the brain tumor?



Microglia in the core and border of the brain metastasis segregate from peri-tumoral microglia



Border and core microglia may have distinct functions



Errede et al., *Fluids and Barriers of the CNS*, 2022 ; Johnson et al., *Journal of Cancer Immunology*, 2020

Summary and Conclusion

- Gene expression is distinct between patient groups

- Microglia function in CNSmets have pro-tumorigenic signature
  - Role of microglia in NonCNSmets is unclear

- Microglia signature in brain metastases are distinct between location
  - Microglia along the border play a role in recruitment

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