Spatial exploration of the tumor microenvironment in renal cell cancer

Practical guide for Visium spatial transcriptomics assays and analysis

Maxime Meylan, Florent Petitprez, Etienne Becht, Antoine Bougouin, Ilenia Giglioli, Virginie Verkarre, Guillaume Lacroix, Johanna Verneau, Chen-Ming Sun, Pierre Laurent-Puig, Yann Vano, Reza Elaïdi, Arnaud Méjean, Rafaël Sanchez-Salas, Eric Barret, Xavier Cathelineau, Stephane Oudard, Claude-Agnès Reynaud, Aurélien de Reyniès, Catherine Sautès-Fridman, Wolf Herman Fridman

Outline

Biological context

Project overview

Practical guide on Visium assays

Bioinformatics methods

Preliminary biological results

B cells and TLS are associated with response to immunotherapy

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B cells are associated with survival and immunotherapy response in sarcoma

Florent Petitprez, Aurélien de Reyniès, [...] Wolf H. Fridman ✉

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B cells and tertiary lymphoid structures promote immunotherapy response

Beth A. Helmink ✉, Sangeetha M. Reddy, [...] Jennifer A. Wargo ✉

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Tertiary lymphoid structures improve immunotherapy and survival in melanoma

Rita Cabrita, Martin Lauss, Adriana Sanna, Marco Donia, Mathilde Skaarup Larsen, Shamik Mitra, Iva Johansson, Bengt Phung, Katja Harbst, Johan Vallon-Christersson, Alison van Schoiack, Kristina Lövgren, Sarah Warren, Karin Jirström, Håkan Olsson, Kristian Pietras, Christian Ingvar, Karolin Isaks: Dirk Schadendorf, Henrik Schmidt, Lars Bastholt, Ana Carneiro, Jennifer A. Wargo, Inge Marie Svane Göran Jönsson ✉

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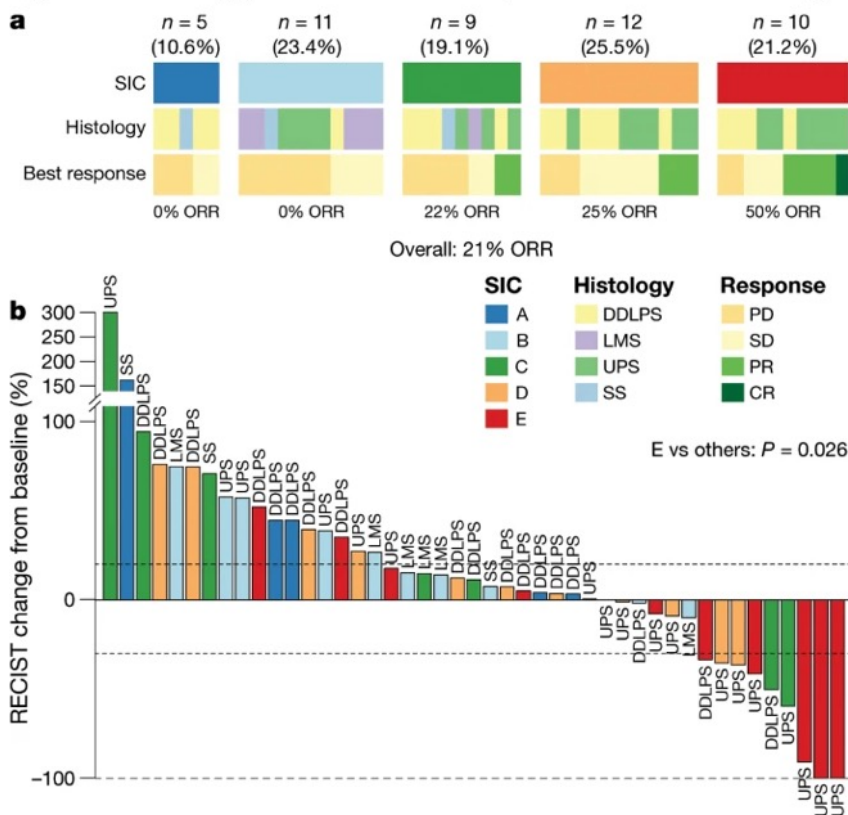
Article | Published: 15 January 2020

B cells are associated with survival and immunotherapy response in sarcoma

Florent Petitprez, Aurélien de Reyniès, Emily Z. Keung, Tom Wei-Wu Chen, Cheng-Ming Sun, Julien Calderaro, Yung-Ming Jeng, Li-Ping Hsiao, Laetitia Lacroix, Antoine Bougoüin, Marco Moreira, Guillaume Lacroix, Ivo Natario, Julien Adam, Carlo Lucchesi, Yec'han Laizet, Maud Toulmonde, Melissa A. Burgess, Vanessa Bolejack, Denise Reinke, Khalid M. Wani, Wei-Lien Wang, Alexander J. Lazar, Christina L. Roland, Jennifer A. Wargo, Antoine Italiano, Catherine Sautès-Fridman, Hussein A. Tawbi ✉ & Wolf H. Fridman ✉ -Show fewer authors

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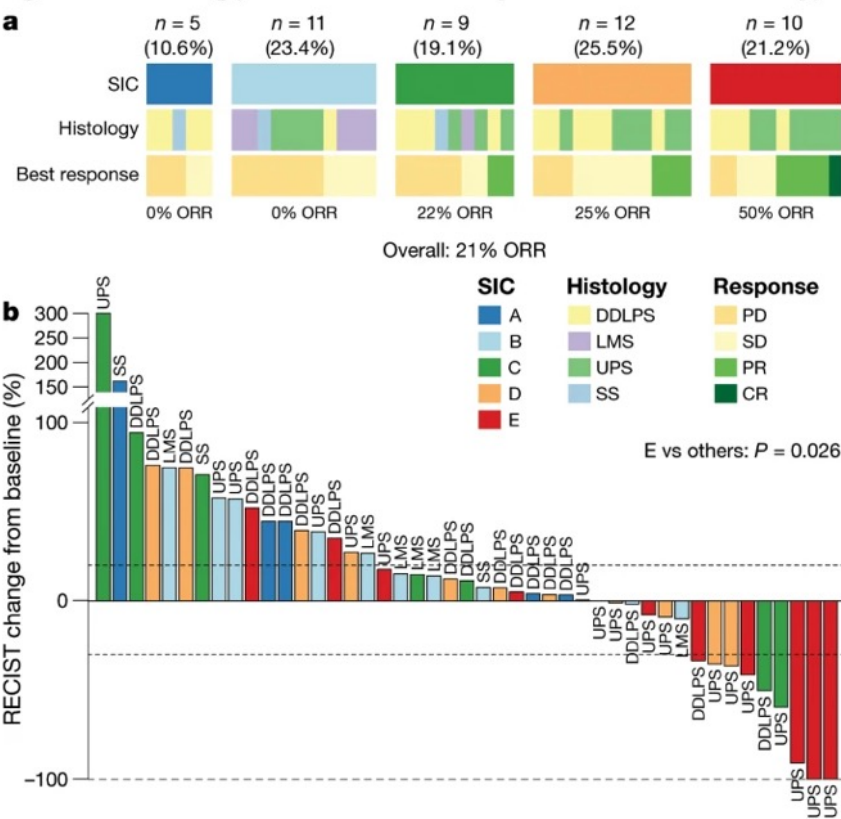Fig. 4: SICs are strongly associated with STS response to PD1 blockade therapy.

TLS are the marker of SIC E

Potential roles of B cells and TLS
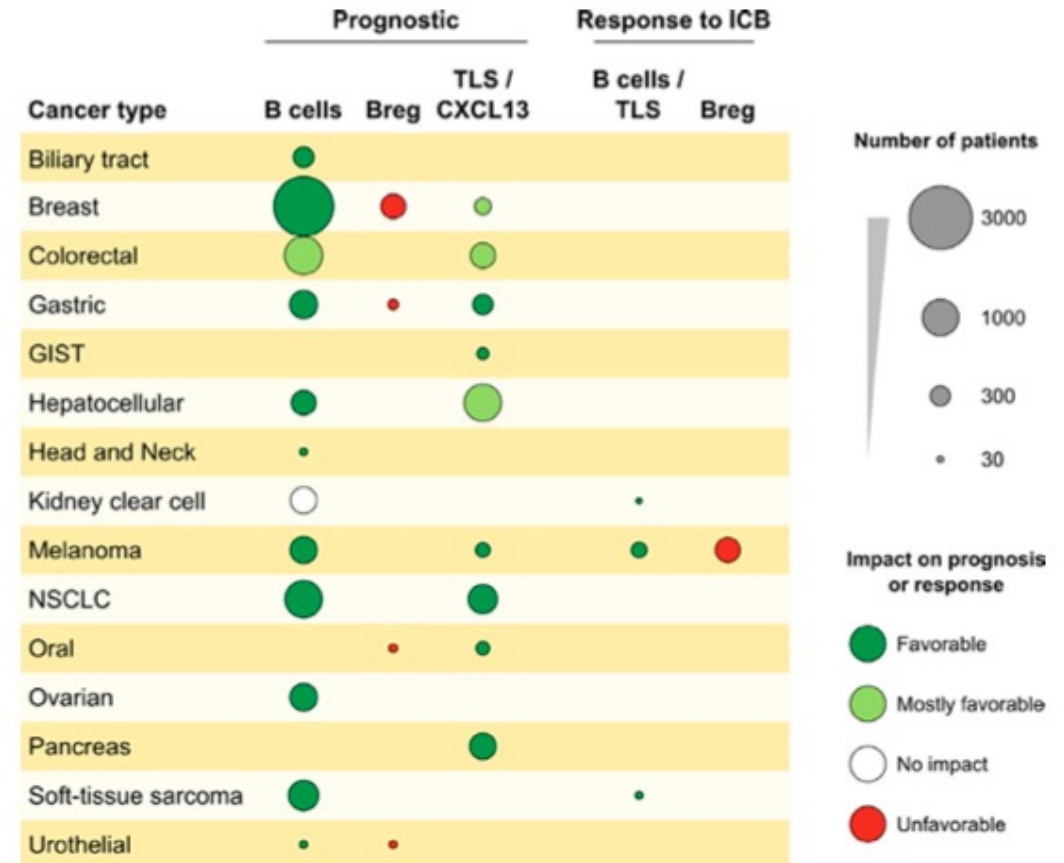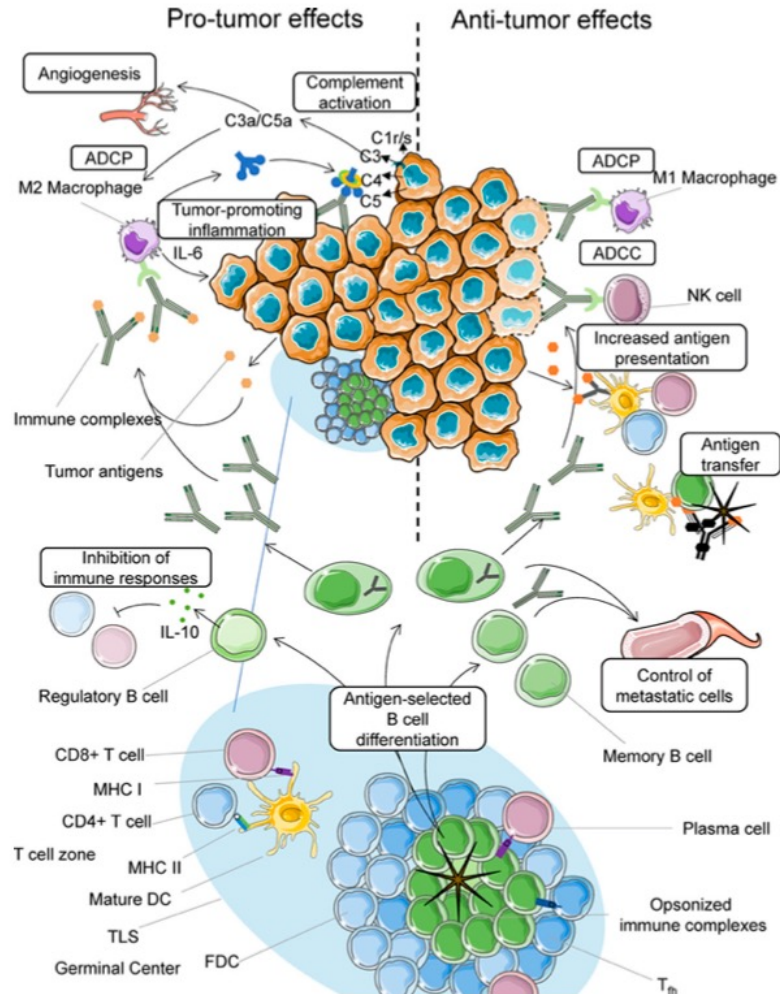
Fridman WH. et al. B cells and cancer: To B or not to B? Journal of Experimental Medicine 2021
Sautès-Fridman C. et al. Tertiary Lymphoid Structures and B cells: Clinical impact and therapeutic modulation in cancer. Seminars in Immunology 2020

In the presence of tertiary lymphoid structures

What is the spatial organization of the TME?
What are the roles of the B cell lineage?
What are the cellular partners of the B cell lineage?
How do TLS and B cells impact the TME?

Visium spatial transcriptomics
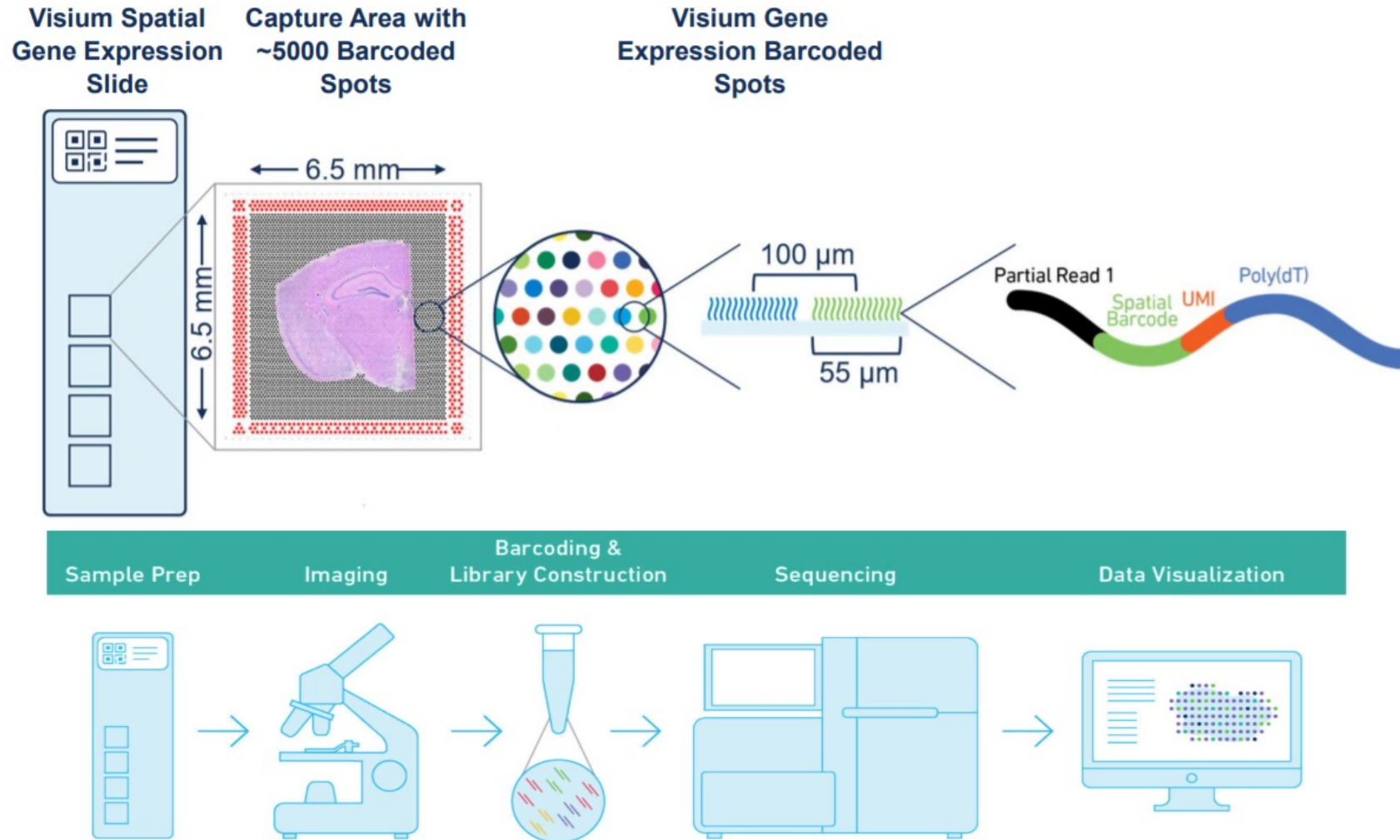
Practical guide on Visium assays

- 1st step : Sample selection

-RNA quality (frozen: RIN > 7 / FFPE: DV200 > 50)

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-Size/shape of the tissue (large, square, compact, low fat/necrosis)

Practical guide on Visium assays

- 1st step : Sample selection

-RNA quality (frozen: RIN > 7 / FFPE: DV200 > 50%)

-Size/shape of the tissue (large, square, compact, low fat/necrosis)

-Test presence of structures of interest (H&E or other stainings)

Data pre-processing

• SpaceRanger pipeline on demultiplexed fatsq's

-Image to data Alignment file (.json)

-Fastqs

-Slide ID and capture area ID

-Reference transcriptome

input

```
cd /home/jdoe/runs
spaceranger count --id=sample345 \
            --transcriptome=/opt/refdata/GRCh38-3.0.0 \
            --fastqs=/home/jdoe/runs/HAWT7ADXX/outs/fastq_path \
            --sample=mysample \
            --image=/home/jdoe/runs/images/sample345.tif \
            --slide=V19J01-123 \
            --area=A1 \
            --loupe-alignment=sample345.json \
            --localcores=8 \
            --localmem=64
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        --localmem=64
```

output

```
Outputs:
- Run summary HTML:                          /opt/sample345/outs/web_summary.html
- Outputs of spatial pipeline:               /opt/sample345/outs/spatial
- Run summary CSV:                           /opt/sample345/outs/metrics_summary.csv
- BAM:                                       /opt/sample345/outs/possorted_genome_bam.bam
- BAM index:                                 /opt/sample345/outs/possorted_genome_bam.bam.bai
- Filtered feature-barcode matrices MEX:     /opt/sample345/outs/filtered_feature_bc_matrix
- Filtered feature-barcode matrices HDF5:    /opt/sample345/outs/filtered_feature_bc_matrix.h5
- Unfiltered feature-barcode matrices MEX:   /opt/sample345/outs/raw_feature_bc_matrix
- Unfiltered feature-barcode matrices HDF5:  /opt/sample345/outs/raw_feature_bc_matrix.h5
- Secondary analysis output CSV:             /opt/sample345/outs/analysis
- Per-molecule read information:             /opt/sample345/outs/molecule_info.h5
- Loupe Browser file:                        /opt/sample345/outs/cloupe.cloupe
- Spatial Enrichment using Moran's I file:   /opt/sample345/outs/spatial_enrichment.csv
```

Data processing with Seurat 4.0.2

- Import "outs" directory with *Load10X_Spatial*
- Filter out spots with high mitochondrial content (> 30%)
→Correlation with necrosis areas
- Remove genes with less than 5 counts
- Remove spots with less than 300 genes

Normalization with SCT on Brain datasets

Normalization and variation stabilisation using regularized negative binomial models
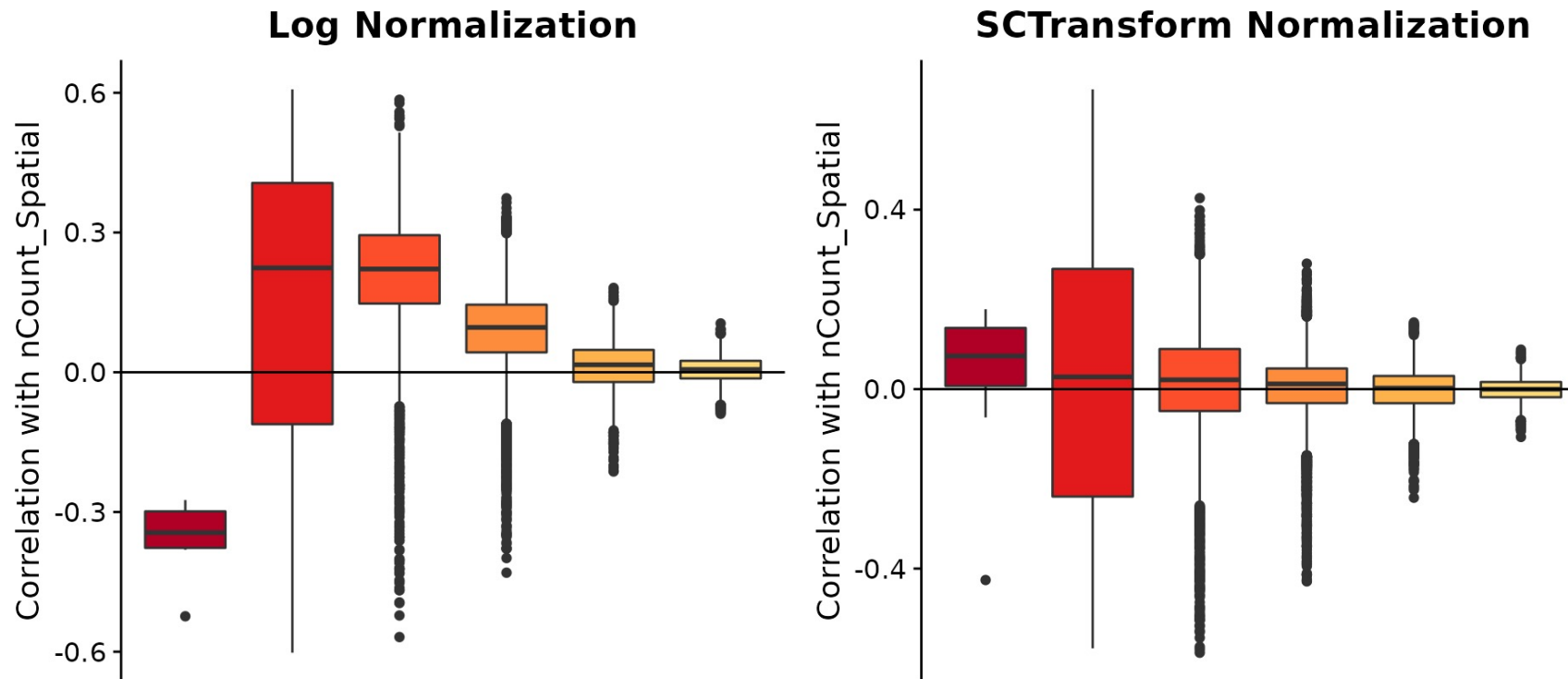
From Seurat vignettes

"We propose that the Pearson residuals from "regularized negative binomial regression," where cellular sequencing depth is utilized as a covariate in a generalized linear model, successfully remove the influence of technical characteristics from downstream analyses while preserving biological heterogeneity."
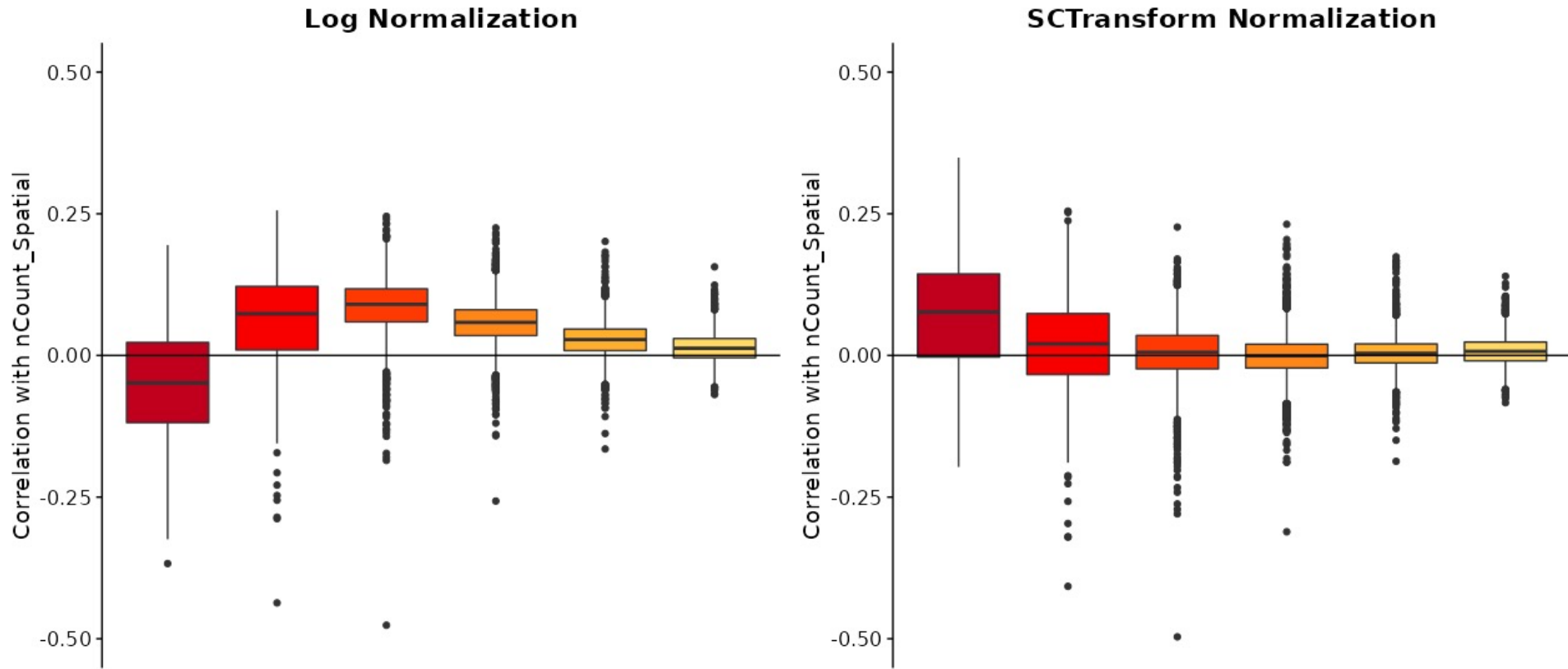
Christoph Hafemeister & Rahul Satija, genome biol 2019
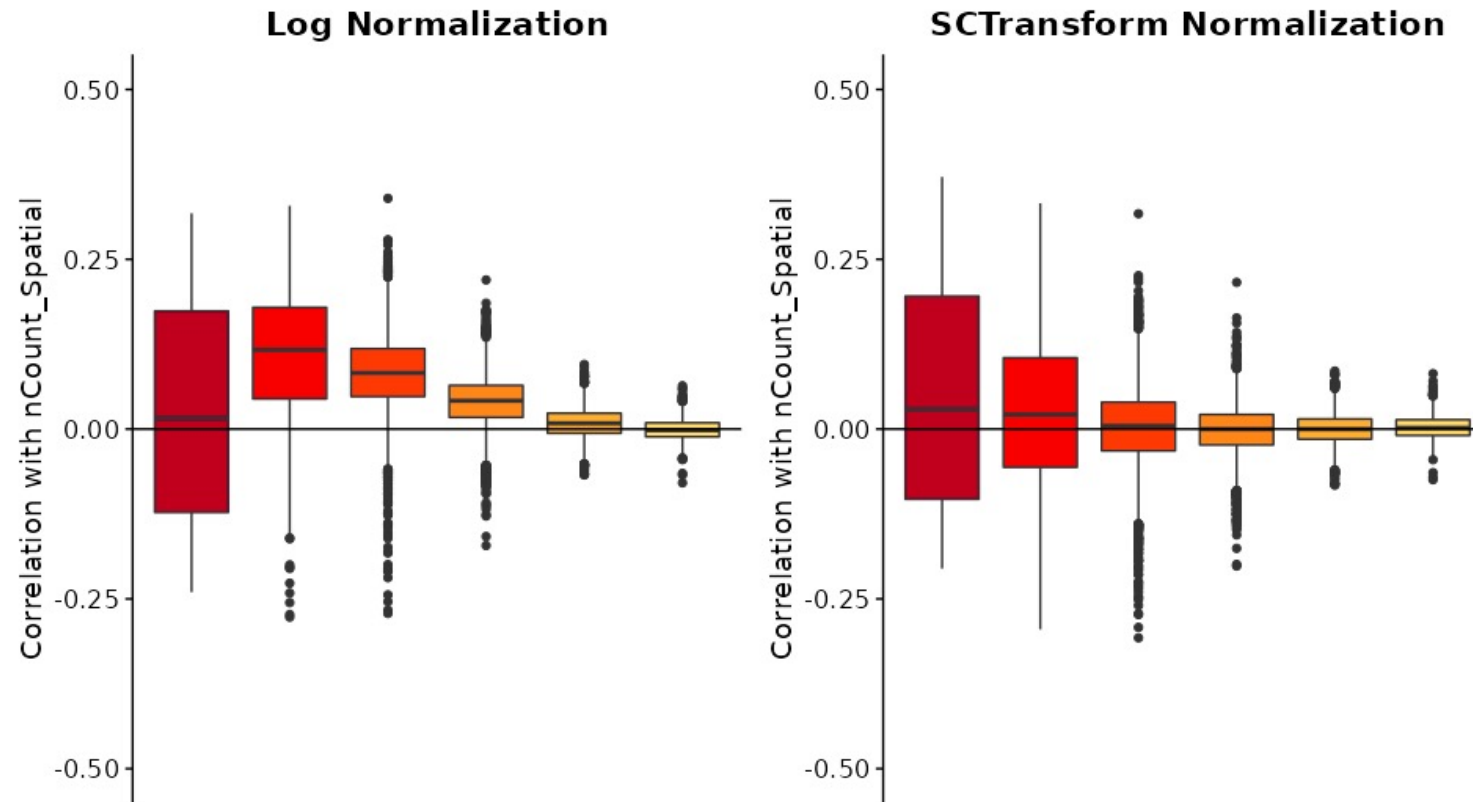
16

Normalization with SCT applied to Frozen sample
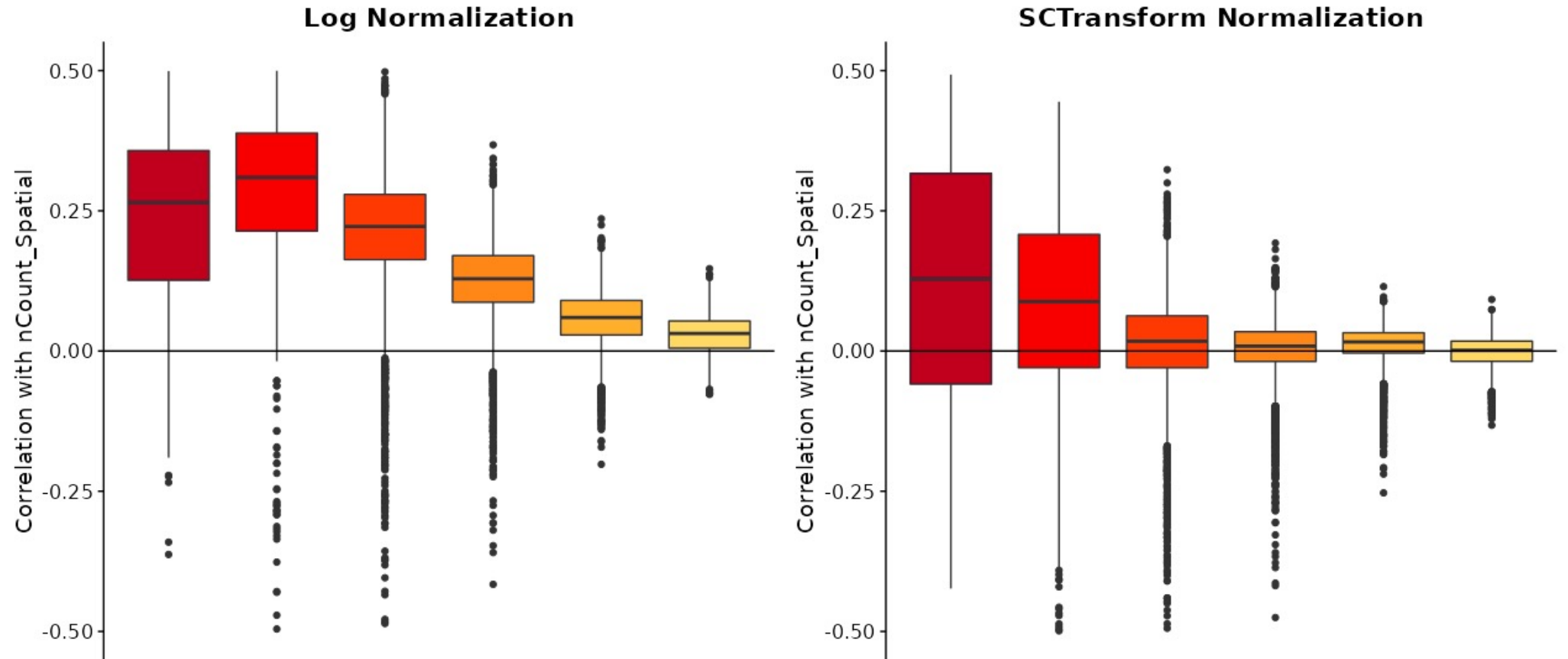
Tumor 1

Normalization with SCT applied to Frozen sample

Tumor 2

Normalization with SCT applied FFPE sample

Tumor 3

Acknowledgments

🐦 **@maxime.meylan**

Cordeliers
- Wolf & Catherine Fridman
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- Guillaume Lacroix
- Chen-Ming Sun

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- Florent Petitprez
- Etienne Becht
- Claude Agnès-Reynaud

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