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Brief Communication

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Integration of whole transcriptome spatial profiling with protein markers

In the format provided by the authors and unedited

Supplementary Note 1:

Assay performance comparisons between SPOTS, Visium-alone, SM-Omics, and Guilliams et al.

SPOTS vs Visium-alone

We examined whether SPOTS and Visium-alone capture comparable RNA signals. By analyzing spleen tissues, we observed higher gene and UMI detection (**Extended Data Fig. 5a**) and less saturated gene expression libraries in Visium-only samples (**Extended Data Fig. 5b**). Nonetheless, these variations had a marginal effect on the overall assay performances, as plotting the detected ADT- UMIs vs RNA- UMIs in defined splenic regions demonstrated a strong positive correlation between the mRNA and protein signals across the major cell-types in spleens, as well as a positive correlation between SPOTS- and Visium-detected genes (**Extended Data Fig. 5c-d**).

We next evaluated the quality of cDNA generated by these methods by processing sequential sections of breast tumor following the Visium or SPOTS protocols. Images of the sections are shown in **Extended Data Figure 8a**. Following the Visium protocol, Visium-only samples were stained for H&E while SPOTS samples were stained for EpCAM. To examine the cDNA quality, we performed qPCR on 11 different housekeeping genes (Qiagen, RT2 Profiler PCR Arrays) and compared the cycle threshold (CT) values in these samples (n = 3 biological replicates, for each). The qPCR results showed modest differences, with Visium samples amplifying on average 1.79 cycles earlier. However, 5 of the 11 genes were found to be expressed at equivalent levels or higher in SPOTS, for example *B2m* and *Tbp*, respectively **(Extended Data Figure 8b)**. Given these results, we concluded that RNA measurements in both methods are approximately equivalent despite differences in fixation and the addition of ADTs in SPOTS, with a marginal advantage to Visium.

We further examined whether gene expression libraries were affected by adding the ADT modality in SPOTS. Here, we performed a comparative analysis between SPOTS and Visium-alone both in different breast cancer tissues (Visium only, n=4; SPOTS, n=3 biological replicates). We measured several parameters including, UMI counts, gene counts, and sequence saturation. As shown in **Extended Data Fig. 8c** and consistent with the cDNA results, we found no evidence for compromised RNA measurements by adding the ADT modality in comparison to Visium-alone, as both methods detected a comparable number of UMIs and genes **(Extended Data Fig. 8c)**. In addition, in sequencing saturation analysis of these samples (SPOTS vs Visium-alone), we found that gene expression libraries in SPOTS were less saturated **(Extended Data Fig. 8d)**, indicating that SPOTS also preserves the library complexity.

Collectively, these data demonstrate a comparable performance of SPOTS with Visium-alone and show that by combining robust protein and mRNA modalities, SPOTS provided more detailed spatial molecular profiles of cellular phenotypes than either modality alone.

SPOTS vs other integrating methodologies

We compared SPOTS' performance with Guilliams et al., (**Extended Data Fig. 5e-g**) and SM-Omics (**Extended Data Fig. 6**), two studies that integrate TotalSeq-A antibodies with ST technology. While SPOTS and Guilliams's methods rely on the Visium platform (55µm spatial barcodes, 100µm center to center distance) and template switched RT-PCR to amplify cDNA, SM-Omics relies on ST slides (100µm spatial barcodes, 200µm center to center distance) and in-vitro transcription to amplify cDNA. We note that beyond the improved performance, SPOTS methodology is also easier to execute, supporting broad applicability. Unlike other multi-omic ST methods that require an automated robotic platform (SM-Omics) or sophisticated microfluidics (DBiT-seq), the SPOTS protocol relies on common lab equipment, which makes it more accessible to a broader spectrum of users.

Comparison of SPOTS to Guilliams et al.

We first compared the total ADT signals in spleen data for SPOTS and liver data for Guilliams et al. Although we found overall similar ADT UMI counts (**Extended Data Fig. 5e**), the ADT signals in Guilliams's study did not construct a coherent data structure (**Extended Data Fig. 5f, correlation map**), as demonstrated by the lack of an expected positive correlation of the same cell type markers, including T-cells (CD3, CD4, CD8), B-cells (CD19, B220, IgD), and macrophages (CD68, F4/80, CD163).

As these datasets were acquired on different tissues, we demonstrate the comparative performance using lineage ADTs that label macrophages (F4/80, CD68, CD163), a cell type that is highly abundant in both tissues. This analysis shows that while in SPOTS data macrophage markers showed high correlation across the spatial barcodes, such correlation was not observed in the Guilliams et al. data (**Extended Data Fig. 5g**).

Comparison of SPOTS to SM-Omics

The comparison to the SM-Omics method is the most relevant, as this method successfully integrated TotalSeq-A antibodies and Visium slides and used the same tissue (mouse spleens). Through this comprehensive analysis (**Extended Data Fig. 6**), we have tested several key parameters, including detection levels of mRNA and ADTs, batch effect, signal correlations, and signal resolution of ADTs and mRNAs.

SPOTS captured a significantly higher number of mRNA across biological replicates. Moreover, SM-Omics exhibited variability in the total ADT counts with lower or comparable capture compared with SPOTS (**Extended Data Fig. 6a**). As both methods characterized the red pulp ("macrophages-enriched" region in SPOTS) and follicular B-cell regions ("B cells-enriched" region in SPOTS), we focused our downstream analyses on ADTs that mark B-cells (IgD) and macrophages (F4/80). Consistent with improved signal capture, SPOTS detected significantly higher numbers of IgD and F4/80 ADTs (**Extended Data Fig. 6b**)**.** Moreover, while in SPOTS the spatial expression of IgD and F4/80 ADTs were mutually excluded with the expected negative correlation of (R = -0.69), in the SM-Omics analysis, these signals were not separated and showed a positive correlation (0.61) (**Extended Data Fig. 6c**). The differences in **panel c** are particularly important as signal separation is essential to construct a biologically meaningful spatial cell clustering.

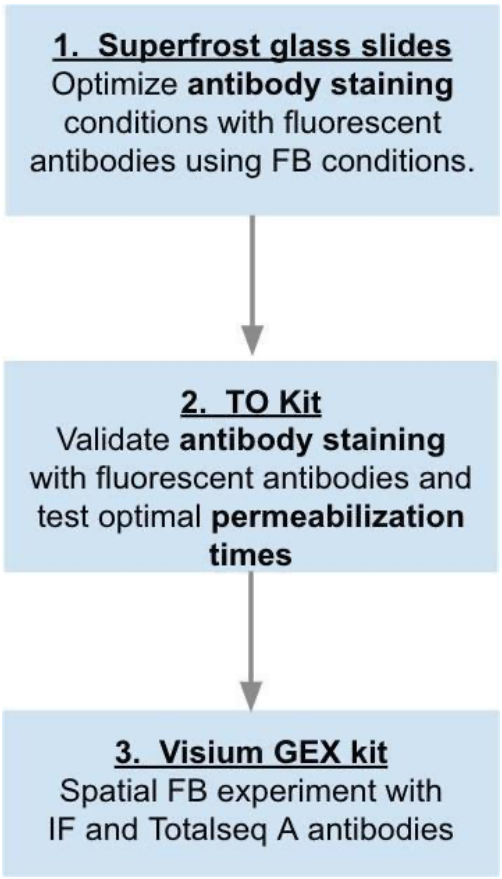
Consequently, the enhanced signal detection in SPOTS translated into improved sensitivity and discriminative power by ADTs (**Extended Data Fig. 6d)** and their corresponding genes (**Extended Data Fig. 6e**), and therefore provided more granular profiling of spatial expression patterns in tissues (**Extended Data Fig. 6f**).

Through these comparative analyses, we demonstrated that SPOTS substantially improved the overall performances of these methodologies in every parameter tested, including signal capture (number of detected genes and ADTs), signal resolution, cell clustering, and discovery power in differential gene expression across tissue regions.

Supplementary Note 2:

Extended SPOTS protocol- Spatial Protein Feature Barcoding on Visium

This protocol should be combined with the Visium Spatial Gene Expression Reagent Kits -Tissue Optimization and Library Preparation User Guides. Before starting the spatial feature barcoding experiment, the user first optimizes immunofluorescence antibody staining conditions on standard glass slides, followed by optimization of permeabilization conditions in combination with immunofluorescence.



1. Optimize immunofluorescence conditions on standard glass slides

TotalSeq A antibody performance

As a first step, we highly recommend verifying the binding specificity of TotalSeq A clones (Biologend) on the tissue of interest by immunofluorescence by using the fluorescently-conjugated clones on standard superfrost glass slides. These steps determine the optimal staining conditions for a select number of markers that act as a proxy for the feature barcoding antibodies. The protocol below outlines the antibody staining in 3X SSC buffer with 2% BSA, 0.1%Tween, and Fc block antibodies (CD16/CD32, Mouse BD Fc Block™; cat 553142).

Notes: The protocol below is optimized for immunofluorescence conditions in the provided slide cassette. Staining conditions or concentrations of these components may be modified for a given set of antibodies/tissue and should be tested by the user. Other components can include but are not limited to, PBS vs TBS-based buffers, blocking solutions (in addition to BSA and Fc block) may include sera or serum components, and detergents such as Triton X-100 or Saponin.

Prepare the following reagents before starting with the workflow:

- Prepare **2x Blocking Buffer**:

	Units	Stock Conc.	Final Conc.	1 Section	X Sections
				Volume (µL)	4X+1
SSC	X	20	6	120.0	
Tween	%	10	0.2	8.0	
BSA	%	10	4	160.0	
Water				112.00	
Total Volume				**400.0**	

- Prepare **Antibody Staining Mix** and keep on ice protected from light until use:

	Units	Stock Conc.	Final Conc.	1 Section	X Sections
				Volume (µL)	4X+1
2X Blocking buffer	X	2	1	25.0	
Fluorescent Antibodies					
Water					
Total Volume				**50.0**	

- Prepare **1x Blocking Solution**:

	Units	Stock Conc.	Final Conc.	1 Section	X Sections
				Volume (µL)	4X+1
2X Blocking Buffer	X	2	1	37.5	
Ribonucleoside Vanadyl Complex*	mM	200	5	1.9	
Fc Block (optional)	-	-	-	5.0	
Water				30.6	
Total Volume				**75.0**	

*Shortly heat the RVC aliquots to 65˚C before use to reconstitute a green-black clear solution.

- Prepare **Wash Buffer**:

	Units	Stock Conc.	Final Conc.	1 Section	X Sections
				Volume (µL)	4X+1
2X Blocking Buffer	X	2	1	300.0	
Water				270.0	
Total Volume				**600.0**	

- Prepare two 50 ml falcon tubes filled with **3X SSC**. **Falcon tube 1** and **Falcon tube 2.**
- **Optional step:** Prepare 1 ml **85% Glycerol or other removable mounting reagent** (prepare only if slide mounting is performed)
- Prepare 10 ml **0.1x SSC**
- Prepare 1 ml **3X SSC**

Workflow

To avoid tissue detachment, the following steps must be performed gently without disturbing the tissue. Tissues are sectioned the day before and kept in -80C

Sectioning and Fixation:

- Section **~10 µm fresh frozen tissue sections** on Tissue Optimization slides as described in the User Guide. Avoid condensation on slide and section. **Keep tissue containing slides on dry ice or -80˚C** without any condensation until proceeding to fixation.
- Prepare falcon tube with freshly prepared **1% Formaldehyde in PBS**.
- Place the slide for **1 minute on a 37˚C heat block**.
- Transfer slide into falcon tube with **1% Formaldehyde**. Make sure all tissue sections are immersed.
- Fix for **10 minutes at RT**.
- Remove excess Formaldehyde by gently tapping the slide corner with Kimwipe.
- Dip slide **5 times** into a **falcon tube 1** filled with 3X SSC.
- Proceed to blocking step and staining.

Visualizing tissue architecture and testing specific antigens

Along with testing clones of interest (fluorescently conjugated), we recommend adding lineage markers with high expression levels in tested tissue (e.g., EpCAM, CD45, PDPN, MHC-II).

Staining with fluorescently conjugated antibodies

- Mount the slide cassette well onto slides without drying the mounted tissues.
- Rehydrate and block by carefully adding **75µl 1X Blocking Solution** into each slide cassette well drop by drop into the corner of the well and **NOT** on the tissue section.
- Block for **15 minutes at 4˚C**.
- Remove Blocking Buffer from wells by slow pipetting.
- Add **50µl Antibody Staining mix** and incubate for **90 minutes at 4˚C** protected from light
- Wash wells 4x with **100µl Wash Buffer** at RT. Incubate 1 min at RT during each wash step.
- Unmount slide from holder and dip 20 times in **falcon tube 2** filled with 3X SSC.

- Carefully remove excess liquid around the tissue with a Kimwipe without drying the tissue section.
- **Optional:** Add 85% glycerol or other removable mounting reagent onto the tissue section and apply cover slip.
  If needed, carefully remove excess mounting medium with Kimwipe.
- Image slides using a fluorescent microscope scanner to capture the whole tissue areas.
- Evaluate the quality of the antibody staining and compare different conditions.

2. Tissue optimization: Validating antibody staining and cDNA

This protocol should be used in combination with the Visium Spatial Tissue Optimization User Guide. The feature barcoding protocol requires different buffers compared to the standard LP protocol. Therefore, the user is required to perform a tissue optimization experiment to test optimal permeabilization conditions. This is performed in combination with immunofluorescent antibody staining to verify that staining does not negatively influence the cDNA yield. The protocol below outlines the antibody staining in 3X SSC buffer with 2% BSA, 0.1% Tween, and Fc block antibodies (CD16/CD32). Other staining buffers and conditions that were established by the user in step 1 of the protocol should be tested in parallel.

Prepare the following reagents before starting with the workflow:

- Prepare **2x Blocking Buffer**:

	Units	Stock Conc.	Final Conc.	1 Section	X Sections
				Volume (µL)	4X+1
SSC	X	20	6	120.0	
Tween	%	10	0.2	8.0	
BSA	%	10	4	160.0	
RNAse Inhibitor	U/µl	20	2	40.0	
Water				72.00	
Total Volume				**400.0**	

- Prepare **Antibody Staining Mix** and keep on ice protected from light until use:

	Units	Stock Conc.	Final Conc.	1 Section	X Sections
				Volume (µL)	4X+1
2X Blocking buffer	X	2	1	25.0	
RNAse Inhibitor (additional)	U/µl	20	1	2.5	
Fluorescent Antibodies					
Water					
Total Volume				**50.0**	

- Prepare **1x Blocking Solution**:

	Units	Stock Conc.	Final Conc.	1 Section	X Sections
				Volume (µL)	4X+1
2X Blocking Buffer	X	2	1	37.5	
Ribonucleoside Vanadyl Complex*	mM	200	5	1.9	
Fc Block	-	-	-	5.0	
Water				30.6	
Total Volume				**75.0**	

*Shortly heat the RVC aliquots to 65˚C before use to reconstitute to a green-black clear solution.

- Prepare **Wash Buffer**:

	Units	Stock Conc.	Final Conc.	1 Section	X Sections
				Volume (µL)	4X+1
2X Blocking Buffer	X	2	1	300.0	
Water				300.0	
Total Volume				**600.0**	

- Prepare **Permeabilization mix (Saponin):**

	1 Section	X Sections
	Volume (µL)	4X+1
10X Saponin	37.5	
RNAse Inhibitor	0.75	
PBS	36.75	
Total Volume	**75.0**	

- Prepare **Mounting Medium (Optional)**:

	1 Section	X Sections
	Volume (µL)	4X+1
Glycerol 85%	225.0	
RNAse Inhibitor	25.0	
Total Volume	**300.0**	

- Prepare two 50 ml falcon tubes filled with **3X SSC**. **Falcon tube 1** and **Falcon tube 2.**
- Prepare 10 ml **0.1x SSC**
- Prepare 1 ml **3X SSC**

Workflow:

Sectioning and Fixation:

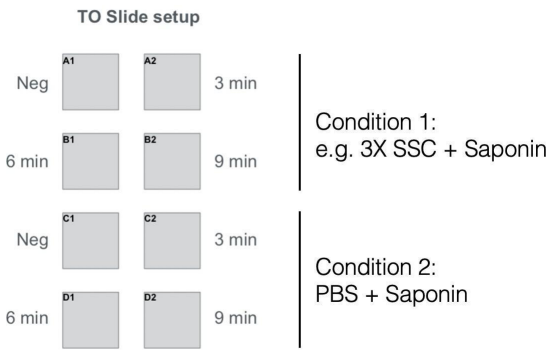
- Section **~10 µm fresh frozen tissue sections** on Tissue Optimization slides as described in the User Guide. Avoid condensation on slide and section. **Keep tissue containing slides on dry ice or -80˚C** without any condensation until proceeding to fixation.
- Prepare falcon tube with freshly prepared 1% Formaldehyde in PBS.
- Place slide for **1 minute on 37˚C heat block**.
- Transfer slide into falcon tube with 1% Formaldehyde. Make sure all tissue sections are immersed.
- Fix for **10 minutes at RT**.
- Remove excess Formaldehyde by tapping the slide corner on Kimwipe.
- Dip slide 5 times into a **falcon tube 1** filled with 3X SSC.
- Proceed to blocking step and staining.

Tissue staining and imaging:

- Mount slides into slide cassettes without drying the slides.
- Rehydrate and block by carefully adding **75µl 1X Blocking Solution** into each slide cassette well drop by drop into the corner of the well and **NOT** on the tissue section.
- Block for **15 minutes at 4˚C**.
- Remove Blocking Buffer and add **75µl Permeabilization mix (Saponin)**
- Incubate for **10 minutes at 4˚C**.
- Remove Permeabilization mix (saponin) from wells by pipetting slowly from the corner of the well.
- Add **50µl Antibody Staining Mix** and incubate for **90 minutes at 4˚C** protected from light
- Wash wells 4x with **100µl Wash Buffer** at RT. Incubate 1 min at RT during each wash step
- Unmount slide from holder and dip 20 times in **falcon tube 2** filled with 3X SSC.
- Carefully remove excess liquid around the tissue with a Kimwipe without drying the tissue section.
- **Optional:** Add ~300 µl mounting medium onto tissue sections and apply cover slip.
    - The large amount of mounting medium ensures that it can be removed easily after imaging without harming the tissue. If needed, carefully remove excess mounting medium with Kimwipe.
- Image slides using fluorescent microscope scanner to capture the whole tissue areas. Evaluate quality of the antibody staining.
- **Optional (only if slides were mounted):** After imaging, immerse the entire slide in a Falcon tube filled with 3X SSC and allow coverslip to detach by gravity.
- Mount slide into slide cassette. Avoid drying the tissue section.
- Wash well once with **100 µl room temperature wash buffer**.
- Wash well once with **100µl room temperature 1X SSC** at room temperature.
- Add **100ul room temperature 1X SSC** to each well.

Permeabilization:

As described in the Tissue Optimization User Guide, test different permeabilization times. 0, 3, 6, and 9 minutes is typically a good range. This allows you to test two different staining conditions or tissues on one TO slide:



- Preheat Thermocycler adapter or Thermomixer to 37˚C
- Prepare **Permeabilization Mix**:

	1 Section	**X Sections**
	Volume (µL)	
Tissue Removal Enzyme (3000387)	3.5	
1X SSC	59.5	
SDS 10%	7.0	
Total Volume	**70.0**	

- Remove SSC and add **70µl of Permeabilization Mix** to each slide cassette well drop by drop onto the center of the tissue section. Tap the cassette gently to spread the mix.
    - Caution: Visually check that Permeabilization Enzyme is evenly covering the entire well.
- Test different permeabilization times: For example, 5, 10, 30 minutes.
- When the incubation is finished, carefully remove the permeabilization mix by pipetting slowly from the corner of the well and wash each well twice with **100µl 0.1X SSC**.
    - Note: The tissue may have entirely disappeared after this step. This does not reduce the performance of the assay!

- **Proceed with Tissue Optimization User Guide at step 2.2: Fluorescent cDNA synthesis.**

3. Library Preparation Workflow

This protocol should be used in combination with the Visium Spatial Gene Expression Reagent Kits User Guide after the optimal permeabilization and antibody staining conditions have been established.

Prepare following reagents before starting with the protocol:

- Prepare **TotalSeqA (BioLegend) antibody panel**:
  When pooling large numbers of antibodies (>20), we recommend exchanging the EDTA containing storage buffer to 3X SSC by centrifuging the pool on 50 kDa cutoff centrifugal filters.
  - Pool the appropriate amounts of TotalSeqA antibodies (typically 0.5 µg antibody per experiment or titrated amounts) to create your panel of interest in a low-bind 1.5 mL tube.
  - Adjust volume to 200 µl with 3X SSC.
  - Pre-wet an Amicon Ultra-0.5 50 kDa MWCO filter unit with 200 µl 3X SSC.
  - Add the antibody pool to the liquid in the filter and spin the unit at 14,000*g* for 4.5 minutes.
  - Discard the flow through and wash by adding 375 µl 3X SSC.
  - Spin the sample for 4 minutes at 14,000*g*. Discard the flow through and collection tube.
  - Invert the filter into a clean collection tube and spin at 1,000*g* for 2 minutes to recover the antibody pool. You will recover a small amount of antibody pool.
  - Use the panel immediately or add 1 µg/µl BSA and 0.06% Sodium Azide for storage at 4˚C.
  - Add fluorescent antibodies according to the antibody staining mix below, **do not** add to the column.

- Prepare **2x Blocking Buffer** as described below or with adjustments determined in steps 1 and 2.

	Units	Stock Conc.	Final Conc.	1 Section	X Sections
				Volume (µL)	4X+1
SSC	X	20	6	120.0	
Tween	%	10	0.2	8.0	
BSA	%	35	4	45.7	
Salmon sperm (sheared)	µg/µl	10	0.2	32.0	
RNAse Inhibitor	U/µl	40	2	40.0	
Water				40.0	
Total Volume				**400.0**	

- Prepare **Antibody Staining Mix** and incubate on ice protected from light at least 30 minutes:

	Units	Stock Conc.	Final Conc.	1 Section	X Sections
				Volume (µL)	4X+1
2X Blocking buffer	X	2.000	1	25.0	
Blocking oligos (dT25)	µM	100	20	10.0	
Fluorescent Antibodies**					
Totalseq A antibody pool					
Water					
Total Volume				**50.0**	

***One antibody fluorophore should be Cy3/PE-labeled to image the fiducial frame in parallel.*

- Prepare **1x Blocking Solution**:

	Units	Stock Conc.	Final Conc.	1 Section	X Sections
				Volume (µL)	4X+1
2X Blocking Buffer	X	2	1	37.5	
Ribonucleoside Vanadyl Complex	mM	200	5	1.9	
Fc Block (optional)	-	-	-	5.0	
Water				30.6	
Total Volume				**75.0**	

* Shortly heat the RVC aliquots to 65˚C before use to reconstitute a green-black clear solution.

- Prepare **Wash Buffer**:

	Units	Stock Conc.	Final Conc.	1 Section	X Sections
				Volume (µL)	4X+1
2X Blocking Buffer	X	2	1	300.0	
Water				300.0	
Total Volume				**600.0**	

* Shortly heat the RVC aliquots to 65˚C before use to reconstitute a green-black clear solution.

- Prepare **Permeabilization mix (Saponin):**

	1 Section	X Sections
	Volume (µL)	4X+1
10X Saponin	37.5	
RNAse Inhibitor	0.75	
PBS	36.75	
Total Volume	**75.0**	

- Prepare **mounting medium (optional):**

	1 Section	X Sections
	Volume (µL)	4X+1
Glycerol 90%	225.0	
RNAse Inhibitor	25.0	
Total Volume	**300.0**	

- Prepare two 50 ml falcon tubes filled with **3X SSC**, **Falcon tube 1** and **Falcon tube 2.**
- Prepare one falcon tube filled with **1X SSC.**
- Prepare 10 ml **0.1X SSC**
- Prepare 10 ml **1X SSC**
- Prepare 10 ml **3XSSC**

Workflow:

Sectioning and Fixation:

- Section **~10 µm fresh frozen tissue sections** on Tissue Optimization slides as described in the User Guide. Avoid condensation on slide and section. **Keep tissue containing slides on dry ice or -80˚C** without any condensation until proceeding to fixation.
- Prepare falcon tube with freshly prepared 1% Formaldehyde in PBS.
- Place the slide for **1 minute on a 37˚C heat block**.
- Transfer slide into falcon tube with 1% Formaldehyde. Make sure all tissue sections are immersed.
- Fix for **10 minutes at RT**.
- Remove excess Formaldehyde by tapping the slide corner on Kimwipe.
- Dip slide 5 times into a **falcon tube 1** filled with 3X SSC.
- Proceed to **blocking step and staining**

Tissue staining and imaging:

- Mount slides into slide cassettes without drying the slides.
- Rehydrate and block by carefully adding **75µl 1X Blocking Solution** into each slide cassette well drop by drop into the corner of the well and **NOT** on the tissue section.
- Block for **15 minutes at 4˚C**.
- Remove Blocking Buffer and add **75µl Permeabilization mix (Saponin)**
- Incubate for **10 minutes at 4˚C**.
- **Place the slide cassette on ice.** Remove Permeabilization mix from wells by pipetting slowly on ice.
- **While the slide cassette is on ice**, add **cold 50µl Antibody Staining Mix** and incubate for **90 minutes at 4˚C** protected from light.
- Wash wells with **100µl Wash Buffer at room temperature**. Incubate 1 min at room temperature during each wash step. Repeat 4x times.
- Unmount slide from holder and dip 20 times in **falcon tube 2** filled with 3X SSC.
- Carefully remove excess liquid around the tissue with a Kimwipe without drying the tissue section.
- **Optional:** Add ~300 µl mounting medium onto tissue sections and apply cover slip.
    - A large volume of mounting medium ensures an easier removal easily after imaging without harming the tissue. If needed, carefully remove excess mounting medium with Kimwipe.
- Image Slide in Fluorescent Microscope or Scanner (AF488/FITC, AF555/PE, AF647/APC).
    - Make sure to image the fiducial frame, in AF555/PE or bright field.
- After imaging. Immerse the entire slide in a Falcon tube filled with **1X SSC** and allow the coverslip to detach by gravity.
- Mount slide into slide cassette. Avoid drying the tissue section.
- Wash well once with **100µl room temperature 1X SSC** at room temperature.
- Add **100ul room temperature 1X SSC** to each well.

Permeabilization:

- Preheat Thermocycler adapter or Thermomixer to 37˚C
- Prepare **Permeabilization Mix**:

	1 Section	X Sections
	Volume (µL)	
Tissue Removal Enzyme (3000387)	3.5	
1X SSC	59.5	
SDS 10%	7.0	
Total Volume	**70.0**	

- Remove 1X SSC and add **70µl of Permeabilization Mix** to each slide cassette well drop by drop onto the center of the tissue section. Tap the cassette gently to spread the mix.
    - Caution: Visually check that Permeabilization Mix is evenly covering the entire well.
- Permeabilize **X minutes** (established during the Tissue Optimization Workflow).
- When the incubation is finished, carefully remove the enzyme by pipetting slowly and wash well **three times** with **150µl 0.1X SSC.**

    - Note: The tissue may have entirely disappeared after this step. This does not reduce performance of the assay!
- Proceed with *Visium Spatial Gene Expression User Guide* at step **1.2 Reverse Transcription** until Second Strand Synthesis.

Second Strand Synthesis (step 3.0):

- Add **2 µl of FB additive primer (100µM)** to the second strand synthesis mix, the final volume of the reaction per section is then 77 µl.
- Proceed as described in protocol until step **4.0 cDNA amplification**.

cDNA Amplification and Cleanup (step 4.0)

- Determine cDNA PCR cycle number as described in the protocol by qPCR.
- After qPCR. Prepare cDNA amplification mix on ice as described in the *Visium Spatial Gene Expression User Guide* (step 4.2) and add FB additive primer to increase yield of Antibody- products:

	1 Sample Volume (µl)	**4 Samples**
Eluted sample	35.0	140.0
Amp Mix (2000047)	51.0	204.0
cDNA Primers (2000089)	15.0	60.0
FB Additive primer (0.2 µM)	1.0	4.0
Total Volume	**102.0**	**408.0**

- Proceed with cDNA amplification as described in *Visium Spatial Gene Expression User Guide* (step 4.2).

cDNA and Antibody-product Size selection

- After cDNA amplification **Antibody-products (~180 bp)** and **mRNA-derived cDNAs (>300 bp)** are separated by 0.6X SPRI. The bead fraction contains mRNA derived cDNAs and supernatant contains ADTs.
    - Add 60 µl SPRI reagent (0.6X) to cDNA reaction.
    - Incubate 5 min at room temperature.
    - Place on the magnet High position and wait ~1 minute until the solution is clear.
    - **Supernatant:** Transfer Supernatant to 1.5 ml low-bind tube **(DO NOT DISCARD! Proceed at Antibody-product cleanup)**.
    - **Beads:** Proceed with cDNA cleanup and library preparation as described in the *Visium Spatial Gene Expression User Guide* (step 4.3).

Antibody-product cleanup

- Antibody-products are purified from highly concentrated cDNA amplification primers by two rounds of 1.9X SPRI cleanups:
    - Add 130 µl SPRI beads to supernatant to obtain a final SPRI to sample ratio of 1.9X.
    - Incubate 5 minutes at room temperature.
    - Place tube on magnet and wait ~2 minutes until solution is clear.
    - Carefully remove and discard the supernatant.

- Add 400 µl 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds (only one Ethanol wash).
- Carefully remove and discard the ethanol wash.
- Centrifuge tube briefly and return it to magnet.
- Remove and discard any remaining ethanol.
- Resuspend in beads in 50 µl water.
- Perform another round of 1.9X SPRI purification by adding 95 µl SPRI reagent directly onto resuspended beads.
- Mix by pipetting and incubate 5 minutes at room temperature.
- Place tube on magnet and wait ~2 minutes until solution is clear.
- Carefully remove and discard the supernatant.
- Add 200 µl 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds (1st Ethanol wash).
- Carefully remove and discard the ethanol wash.
- Add 200 µl 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds (2nd Ethanol wash).
- Carefully remove and discard the ethanol wash.
- Centrifuge tube briefly and return it to magnet.
- Remove and discard any remaining ethanol and allow the beads to air dry for 2 minutes (do not over dry beads).
- Resuspend beads in 45 µl water.
- Pipette mix vigorously and incubate at room temperature for 5 minutes.
- Place tube on magnet and transfer clear supernatant into PCR tube.

FB Antibody Sequencing Library Amplification

- Prepare 100µl PCR reaction of purified ADTs:

	1 sample Volume (µl)	4 samples
Purified Antibody-product fraction	45.0	
Amp Mix (2000047)	50.0	
TruSeq Small RNA RPIx primer (20 µM)	2.5	
SI-PCR primer (20 µM)	2.5	
Total Volume:	**100.0**	

Cycling conditions:

95˚C	3 min	
95˚C	20 sec	|
60˚C	30 sec	|~ 6-10 cycles
72˚C	20 sec	|
72˚C	5 min	

- Purify Antibody PCR product **1X SPRI**:
  - Add 100 µl SPRI reagent to sample.
  - Incubate 5 minutes at room temperature.
  - Place tube on magnet High position and wait 1 minute until solution is clear.
  - Carefully remove and discard the supernatant.
  - Add 200 µl 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds (first Ethanol wash).
  - Carefully remove and discard the ethanol wash.
  - Add 200 µl 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds (second Ethanol wash).
  - Carefully remove and discard the ethanol wash.
  - Centrifuge tube briefly and return it to magnet.
  - Remove any remaining ethanol and allow the beads to air dry for 2 minutes.
  - Resuspend beads in 30 µl water.
  - Pipette mix vigorously and incubate at room temperature for 5 minutes.
  - Place tube on magnet and transfer clear supernatant to PCR tube.
  - Quantify libraries by standard methods (QuBit, BioAnalyzer, qPCR).
    - Antibody libraries will be ~180 bp
  - An**tibody libraries are now ready to be sequenced**

Materials and reagents needed

All reagents should be RNAse free!

Reagent	Notes	Vendor / Order Number
Formaldehyde solution (~37%)	Can also use methanol-free FA ampules	Sigma #F8775-25ML
BSA (protease, nuclease free)	Prepare 10% Aliquots	VWR #0332-25K
Sheared Salmon Sperm		Invitrogen 310249194
Ribonucleoside Vanadyl Complexes	Prepare single use frozen aliquots. Sensitive to EDTA.	Sigma #R3380-5ML
Fc Block (CD16/CD32) mAb	Must be species specific	e.g. BD Biosciences
Amicon Ultra-0.5 Centrifugal Filter Unit	50kDa cutoff	EMD Milipore #UFC5050
20X SSC		e.g. Sigma
Tween20		e.g. Sigma
10X SDS		e.g. Sigma
Triton X		e.g. Sigma
Glycerol		e.g. Sigma
X10 Saponin	0.5% w/v soln. in PBS (10X)	Thermo fisher #J63209.AK
RNAse Inhibitor	Recommended Roche Protector RNAse Inhibitor	Roche
Apex Superior Adhesive slides (white)		Leica #3800080
Coverslips		Leica

Oligos	Sequence	Index	Stock concentration
dT25_block	TTTTTTTTTTTTTTTTTTTTTTTT*T*T*/3InvdT/	-	100uM
FB additive primer	CCTTGGCACCCGAGAATT*C*C*A	-	100uM and 0.2uM
SI-PCR	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTC	-	20uM
RPI_B1	CAAGCAGAAGACGGCATACGAGAT AAGATTAC GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA	GTAATCTT	20uM
RPI_B2	CAAGCAGAAGACGGCATACGAGAT GAAGAGTA GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA	TACTCTTC	20uM
RPI_B3	CAAGCAGAAGACGGCATACGAGAT TAATACAC GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA	GTGTATTA	20uM
RPI_B4	CAAGCAGAAGACGGCATACGAGAT TATGAAGT GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA	ACTTCATA	20uM
RPI_B5	CAAGCAGAAGACGGCATACGAGAT CCATTATT GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA	AATAATGG	20uM
RPI_B6	CAAGCAGAAGACGGCATACGAGAT GATTAACG GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA	CGTTAATC	20uM
RPI_B7	CAAGCAGAAGACGGCATACGAGAT TGAGGTTT GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA	AAACCTCA	20uM
RPI_B8	CAAGCAGAAGACGGCATACGAGAT AGCACTTT GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA	AAAGTGCT	20uM
RPI_B9	CAAGCAGAAGACGGCATACGAGAT AGTTACAG GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA	CTGTAACT	20uM
RPI_B10	CAAGCAGAAGACGGCATACGAGAT CATACGGT GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA	ACCGTATG	20uM
RPI_B11	CAAGCAGAAGACGGCATACGAGAT TGAGGAAC GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA	GTTCCTCA	20uM
RPI_B12	CAAGCAGAAGACGGCATACGAGAT TGGTGGTA GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA	TACCACCA	20uM
	*These indexes were taken from SI-GA-B indexes and are compatible with all other SI-GA- index plate rows except SI-GA-B row for cDNA libraries.		

Supplementary Note 3: SPOTS analysis of murine breast cancer

Steve X. Niu[1]

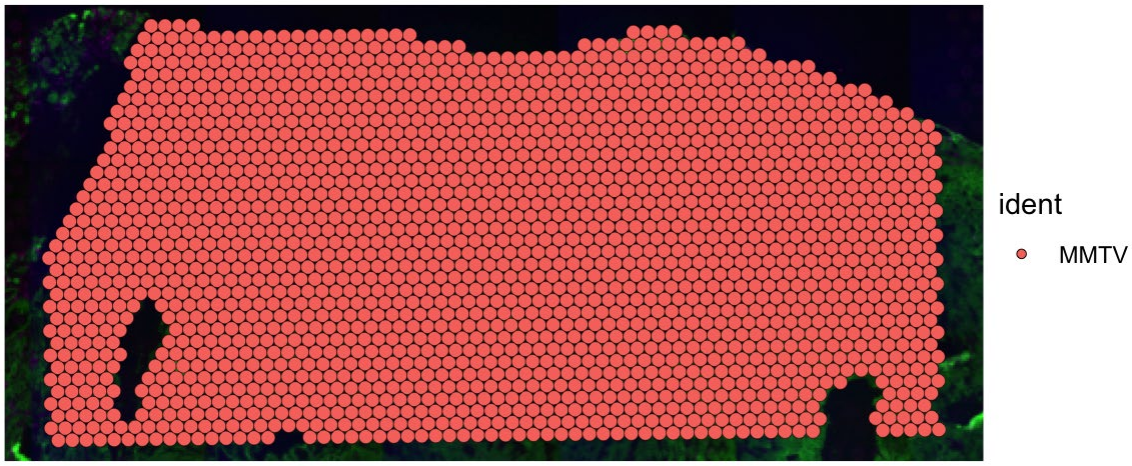
Last Run: July 29, 2022

1. Load Data This markdown file contains the scRNA-seq analysis for the paper titled **Integrated protein and transcriptome high-throughput spatial profiling**. The data used for this analysis can be found under the GEO repository GSE198353.

```
# install Seurat v4.0.0 and spots
if (!requireNamespace("remotes", quietly = TRUE))
  install.packages("remotes")
if (!requireNamespace("Seurat", quietly = TRUE) | utils::packageVersion("Seurat") < "4.0.0")
  remotes::install_version("Seurat", version = "4.0.0")
if (!requireNamespace("spots", quietly = TRUE))
  install.packages("spots")

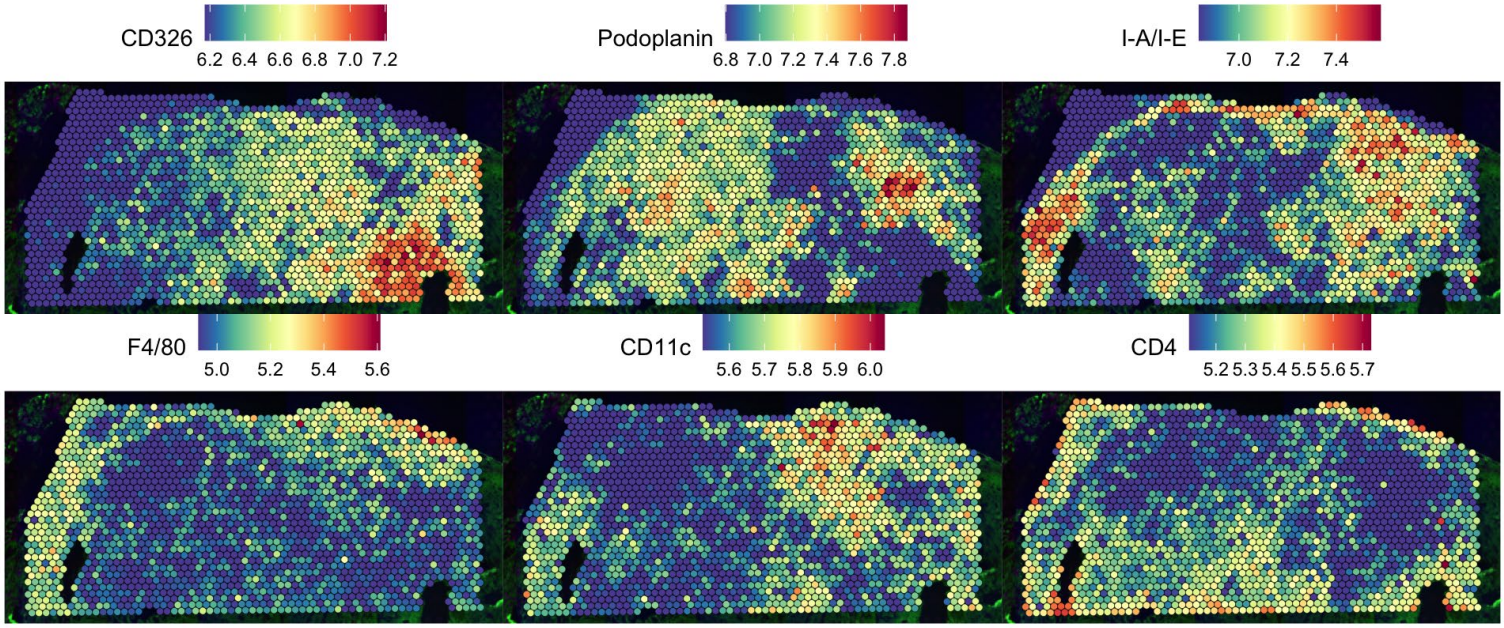
# load data
library(Seurat)
library(spots)

mmtv_gex <- Read10X_h5('GSE198353_mmtv_pymt_GEX_filtered_feature_bc_matrix.h5')
mmtv_adt <- read.csv('GSE198353_mmtv_pymt_ADT.csv.gz', header = TRUE, row.names = 1, check.nam
es = FALSE)
mmtv_image <- Read10X_Image('GSE198353_mmtv_pymt_spatial')
mmtv <- CreateSeuratObject(mmtv_gex, assay = "RNA", project = "MMTV")
mmtv_adt <- CreateSeuratObject(mmtv_adt, assay = "CITE", project = "MMTV")
mmtv@assays$CITE <- mmtv_adt@assays$CITE
mmtv$nCount_CITE <- mmtv_adt$nCount_CITE
mmtv$nFeature_CITE <- mmtv_adt$nFeature_CITE
mmtv_image@key <- "A"
mmtv@images <- list(A = mmtv_image)
SpatialDimPlot(mmtv)
```



2. Data normalization

```
mmtv <- NormalizeData(mmtv, assay = "RNA", verbose = FALSE)
mmtv <- NormalizeData(mmtv, assay = "CITE", verbose = FALSE)
DefaultAssay(mmtv) <- "CITE"
mmtv <- ScaleData(mmtv, verbose = FALSE)
SpatialFeaturePlot(mmtv,features = c("CD326","Podoplanin","I-A/I-E",
                                     "F4/80", "CD11c","CD4"), ncol = 3, min.cutoff = "q25")
```



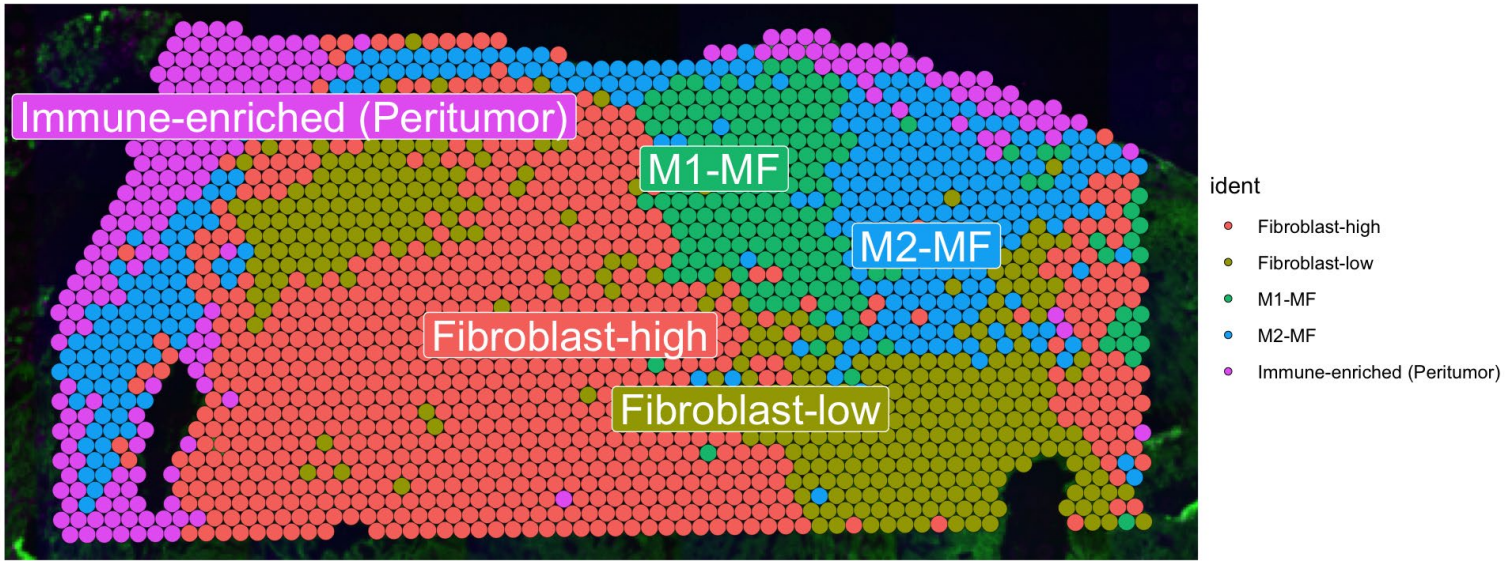
2. Spatial Component Analysis and Clustering

```
Visium.hnn.dist <- LoadData("~/Downloads", "Visium.HNN")
mmtv.hnn.dist <- VisiumHnn("~/Downloads/", Cells(mmtv))
mmtv.hnn <- HnnNeighbor(mmtv.hnn.dist, k = 37, include.self = FALSE)
mmtv.hnn.weight <- HnnWeight(mmtv.hnn$dist.mat, dist.k = 3, sigma = 1)
mmtv.sca <- SCA(X = Matrix::t(mmtv@assays$CITE@data),
                W = mmtv.hnn.weight,
                scaled.data = t(mmtv@assays$CITE@scale.data),
                n.eigen = 30)
mmtv@reductions[["sca"]] <- CreateDimReducObject(embeddings = mmtv.sca$X,
                                                 loadings = mmtv.sca$rotation,
                                                 stdev = mmtv.sca$eigenvalues,
                                                 key = "SC_", assay = "CITE")
mmtv <- FindNeighbors(mmtv, reduction = "sca", dims = 1:15)
mmtv <- FindClusters(mmtv, resolution = 0.3)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
```

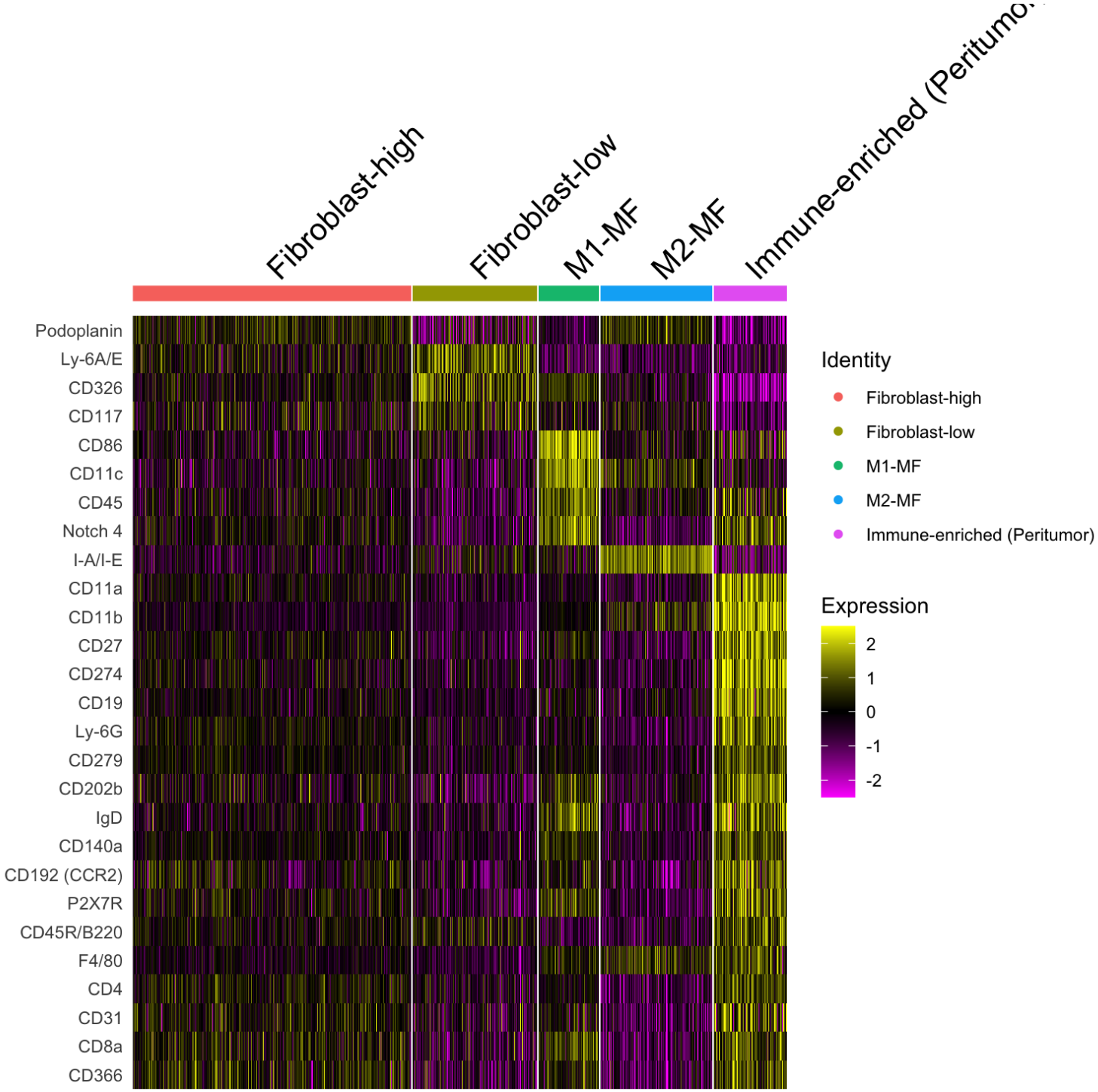
```
##
## Number of nodes: 1978
## Number of edges: 72438
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8278
## Number of communities: 5
## Elapsed time: 0 seconds
```

```
mmtv <- RenameIdents(mmtv, '0' = 'Fibroblast-high',
                     '1' = 'Fibroblast-low',
                     '2' = 'M2-MF',
                     '3' = 'Immune-enriched (Peritumor)',
                     '4' = 'M1-MF')
mmtv@active.ident = factor(mmtv@active.ident, levels = c('Fibroblast-high', 'Fibroblast-low','
M1-MF',
                                                         'M2-MF','Immune-enriched (Peritumor)'
))
SpatialDimPlot(mmtv, label = TRUE, repel = TRUE)
```



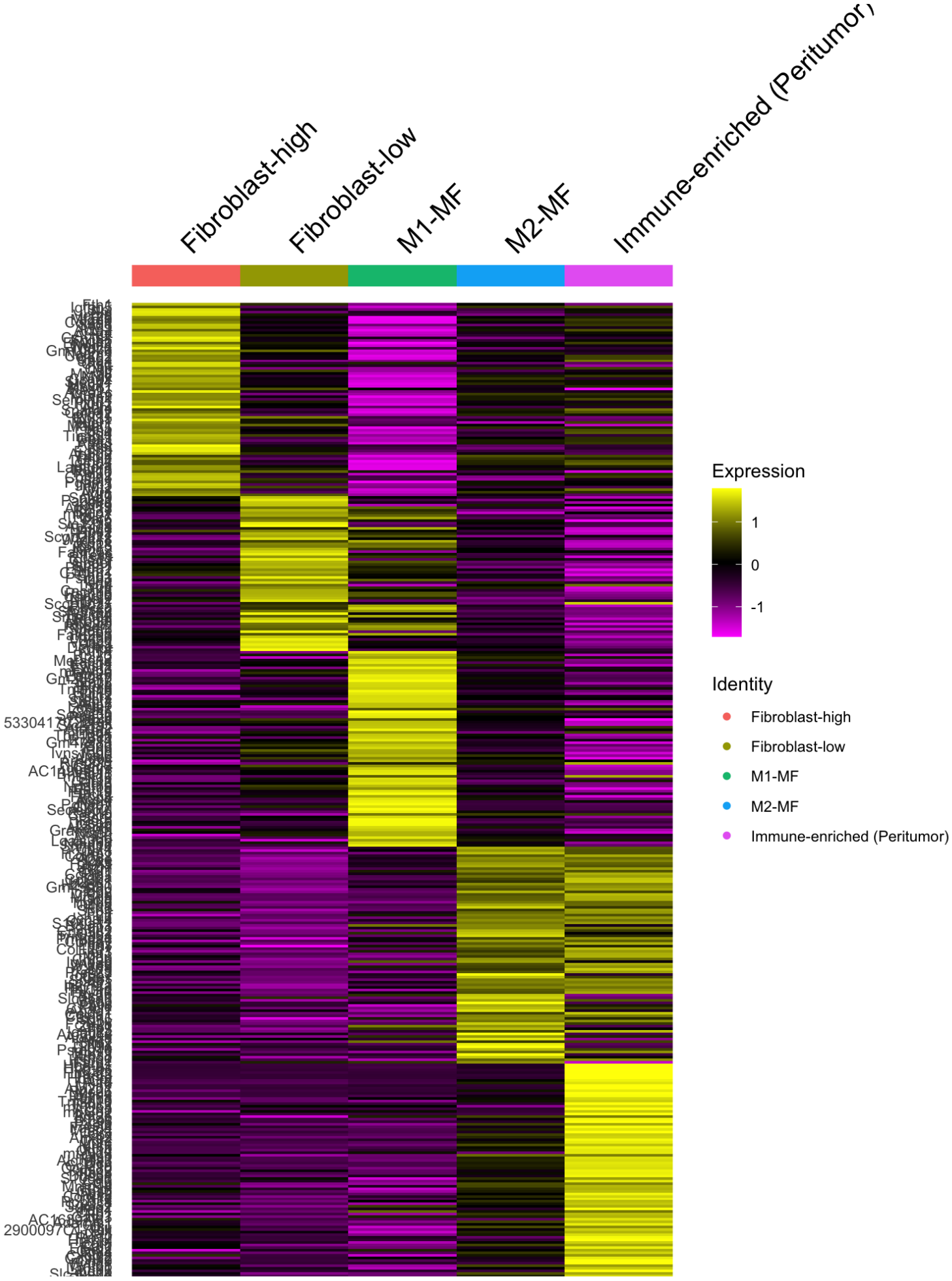
3. Differentially expressed ADTs

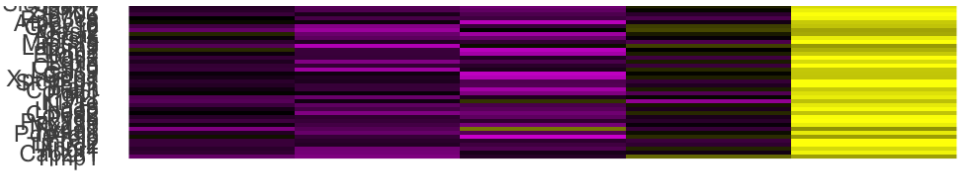
```
adt.markers <- FindAllMarkers(mmtv, only.pos = TRUE, logfc.threshold = 0.2, verbose = FALSE)
DoHeatmap(mmtv, adt.markers$gene, assay = "CITE", angle = 45)
```



4. Differentially expressed mRNAs

```
DefaultAssay(mmtv) = "RNA"
mrna.markers <- FindAllMarkers(mmtv, only.pos = TRUE, verbose = FALSE)
mmtv.avg <- AverageExpression(mmtv, assays = "RNA", return.seurat = TRUE)
DoHeatmap(mmtv.avg, mrna.markers$gene, draw.lines = FALSE, assay = "RNA", angle = 45)
```


Fibroblast-high
Fibroblast-low
M1-MF
M2-MF
Immune-enriched (Peritumor)
Expression
1
0
-1
Identity
Fibroblast-high
Fibroblast-low
M1-MF
M2-MF
Immune-enriched (Peritumor)



5. Session info

```
# Session info
print(sessionInfo())
```

```
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur 10.16
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRblas.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] spots_0.1.0        SeuratObject_4.0.0 Seurat_4.0.1
##
## loaded via a namespace (and not attached):
##   [1] Rtsne_0.15            colorspace_2.0-0      deldir_0.2-3          ellipsis_0.3.2
   ggridges_0.5.3        rstudioapi_0.13
##   [7] spatstat.data_1.7-0   farver_2.0.3          leiden_0.3.6          listenv_0.8.0
   remotes_2.4.2         bit64_4.0.5
##  [13] ggrepel_0.9.0         RSpectra_0.16-0       codetools_0.2-18      splines_4.0.3
   knitr_1.38            polyclip_1.10-0
##  [19] jsonlite_1.7.2        ica_1.0-2             cluster_2.1.0         png_0.1-7
   uwot_0.1.10           shiny_1.5.0
##  [25] sctransform_0.3.2     spatstat.sparse_2.0-0 compiler_4.0.3        httr_1.4.2
   Matrix_1.3-2          fastmap_1.1.0
##  [31] lazyeval_0.2.2        limma_3.46.0          cli_3.2.0             later_1.1.0.1
   htmltools_0.5.2       tools_4.0.3
##  [37] igraph_1.2.6          gtable_0.3.0          glue_1.6.2            RANN_2.6.1
   reshape2_1.4.4        dplyr_1.0.2
##  [43] Rcpp_1.0.7            scattermore_0.7       jquerylib_0.1.3       vctrs_0.4.1
   nlme_3.1-151          lmtest_0.9-38
##  [49] xfun_0.30             stringr_1.4.0         globals_0.14.0        mime_0.9
   miniUI_0.1.1.1        lifecycle_1.0.1
##  [55] irlba_2.3.3           goftest_1.2-2         future_1.21.0         MASS_7.3-53
   zoo_1.8-8             scales_1.1.1
##  [61] spatstat.core_1.65-5  promises_1.1.1        spatstat.utils_2.1-0  parallel_4.0.3
   RColorBrewer_1.1-2    yaml_2.2.1
##  [67] reticulate_1.18       pbapply_1.4-3         gridExtra_2.3         ggplot2_3.3.3
   sass_0.4.0            rpart_4.1-15
```

```
##  [73] stringi_1.5.3         highr_0.8             rlang_1.0.2           pkgconfig_2.0.3
   matrixStats_0.57.0    evaluate_0.15
##  [79] lattice_0.20-41       ROCR_1.0-11           purrr_0.3.4           tensor_1.5
   labeling_0.4.2        patchwork_1.1.1
##  [85] htmlwidgets_1.5.3     bit_4.0.4             cowplot_1.1.1         tidyselect_1.1.0
   parallelly_1.23.0     RcppAnnoy_0.0.18
##  [91] plyr_1.8.6            magrittr_2.0.1        R6_2.5.0              generics_0.1.0
   DBI_1.1.0             mgcv_1.8-33
##  [97] pillar_1.4.7          fitdistrplus_1.1-3    survival_3.2-7        abind_1.4-5
   tibble_3.0.4          future.apply_1.7.0
## [103] hdf5r_1.3.5           crayon_1.3.4          KernSmooth_2.23-18    spatstat.geom_1.65-
5  plotly_4.9.2.2        rmarkdown_2.13
## [109] grid_4.0.3            data.table_1.13.6     digest_0.6.27         xtable_1.8-4
   tidyr_1.1.2           httpuv_1.5.4
## [115] munsell_0.5.0         viridisLite_0.3.0     bslib_0.3.1
```

1. Tri-Institutional Training Program in Computational Biology and Medicine, xin2001@med.cornell.edu↩

Supplementary Note 4: SPOTS analysis of murine spleen

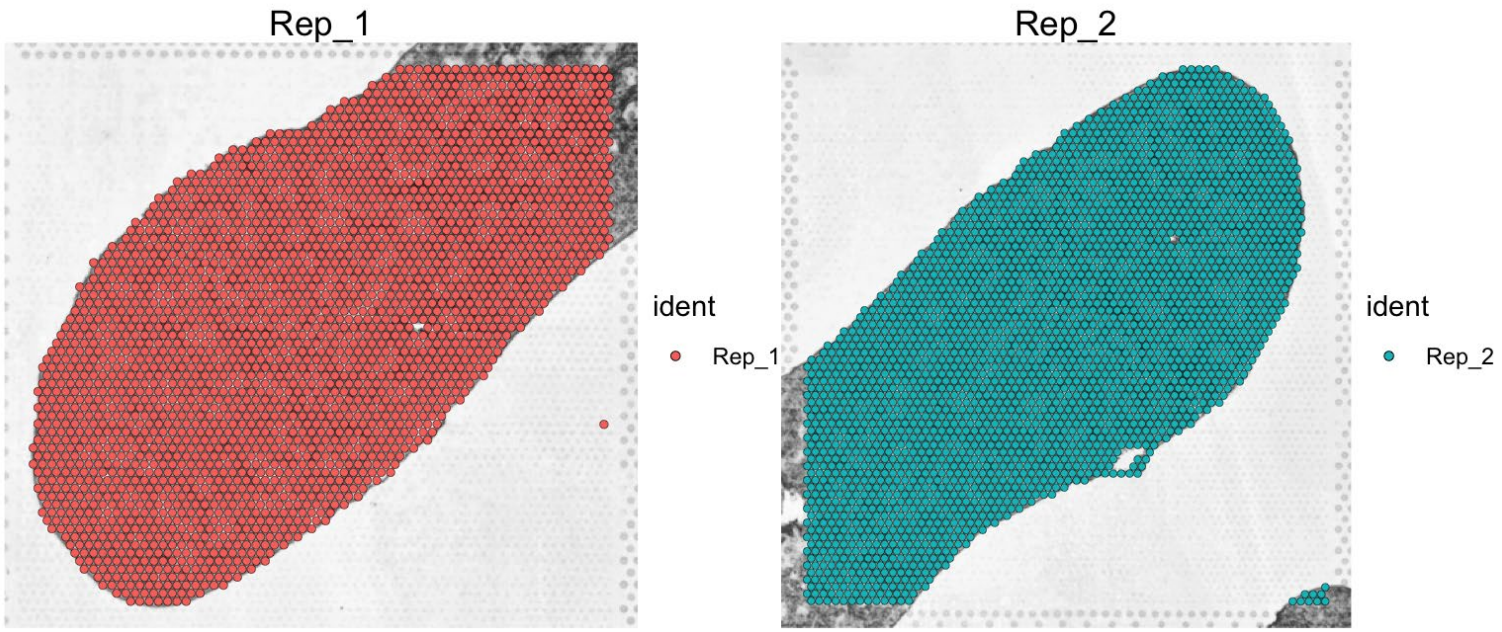
Steve X. Niu[1]

Last Run: March 21, 2022

1. Load Data This markdown file contains the scRNA-seq analysis for the paper titled **Integrated protein and transcriptome high-throughput spatial profiling**. The data used for this analysis can be found under the GEO repository GSE198353.

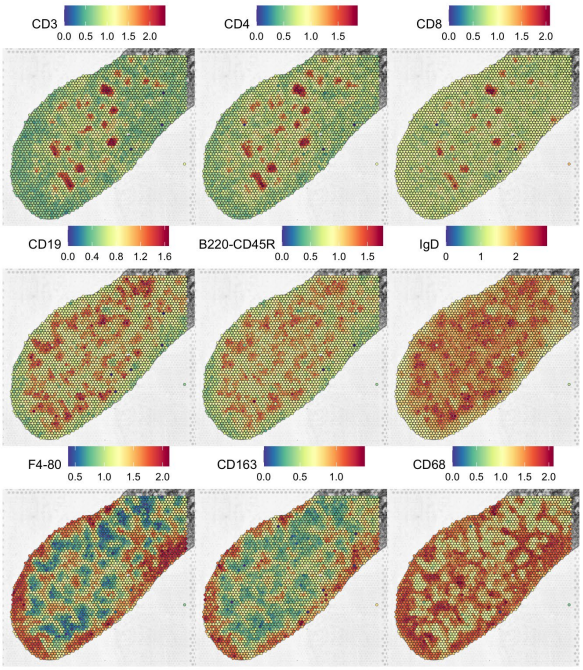
```
# install Seurat v3.0.0
if (!requireNamespace("Seurat", quietly = TRUE) | utils::packageVersion("Seurat") < "4.0.0")
  remotes::install_version("Seurat", version = "4.0.0")

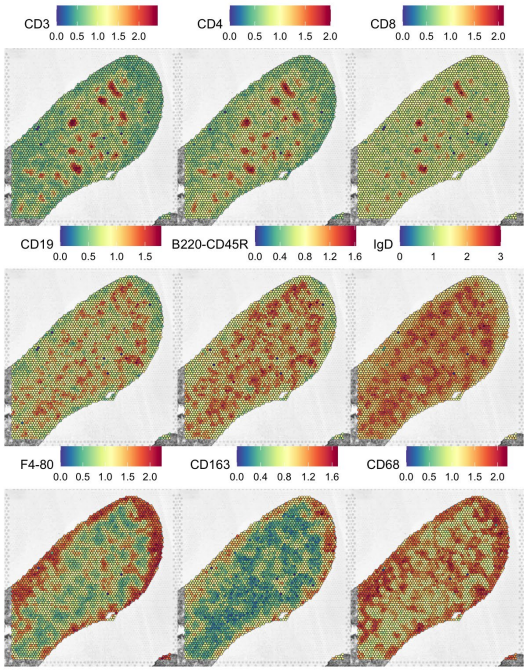
# load data
library(Seurat)
# replicate 1
rep_1_data <- Read10X_h5('spleen_rep_1_filtered_feature_bc_matrix.h5')
rep_1_image <- Read10X_Image('GSE198353_spleen_replicate_1_spatial')
rep_1 <- CreateSeuratObject(rep_1_data$`Gene Expression`, assay = "RNA", project = "Rep_1")
rep_1_CITE <- CreateSeuratObject(rep_1_data$`Antibody Capture`, assay = "CITE", project = "Rep
_1")
rep_1@assays$CITE <- rep_1_CITE@assays$CITE
rep_1$nCount_CITE <- rep_1_CITE$nCount_CITE
rep_1$nFeature_CITE <- rep_1_CITE$nFeature_CITE
rep_1_image@assay <- c("RNA", "CITE")
rep_1_image@key <- "Rep_1"
rep_1@images <- list(Rep_1 = rep_1_image)
# replicate 2
rep_2_data <- Read10X_h5('spleen_rep_2_filtered_feature_bc_matrix.h5')
rep_2_image <- Read10X_Image('GSE198353_spleen_replicate_2_spatial')
rep_2 <- CreateSeuratObject(rep_2_data$`Gene Expression`, assay = "RNA", project = "Rep_2")
rep_2_CITE <- CreateSeuratObject(rep_2_data$`Antibody Capture`, assay = "CITE", project = "Rep
_2")
rep_2@assays$CITE <- rep_2_CITE@assays$CITE
rep_2$nCount_CITE <- rep_2_CITE$nCount_CITE
rep_2$nFeature_CITE <- rep_2_CITE$nFeature_CITE
rep_2_image@assay <- c("RNA", "CITE")
rep_2_image@key <- "Rep_2"
rep_2@images <- list(Rep_2 = rep_2_image)
# merge
spleen <- merge(rep_1, rep_2, add.cell.ids = c("Rep_1", "Rep_2"), project = "Spleen")
SpatialDimPlot(spleen, images = c("Rep_1", "Rep_2"))
```



2. Data normalization

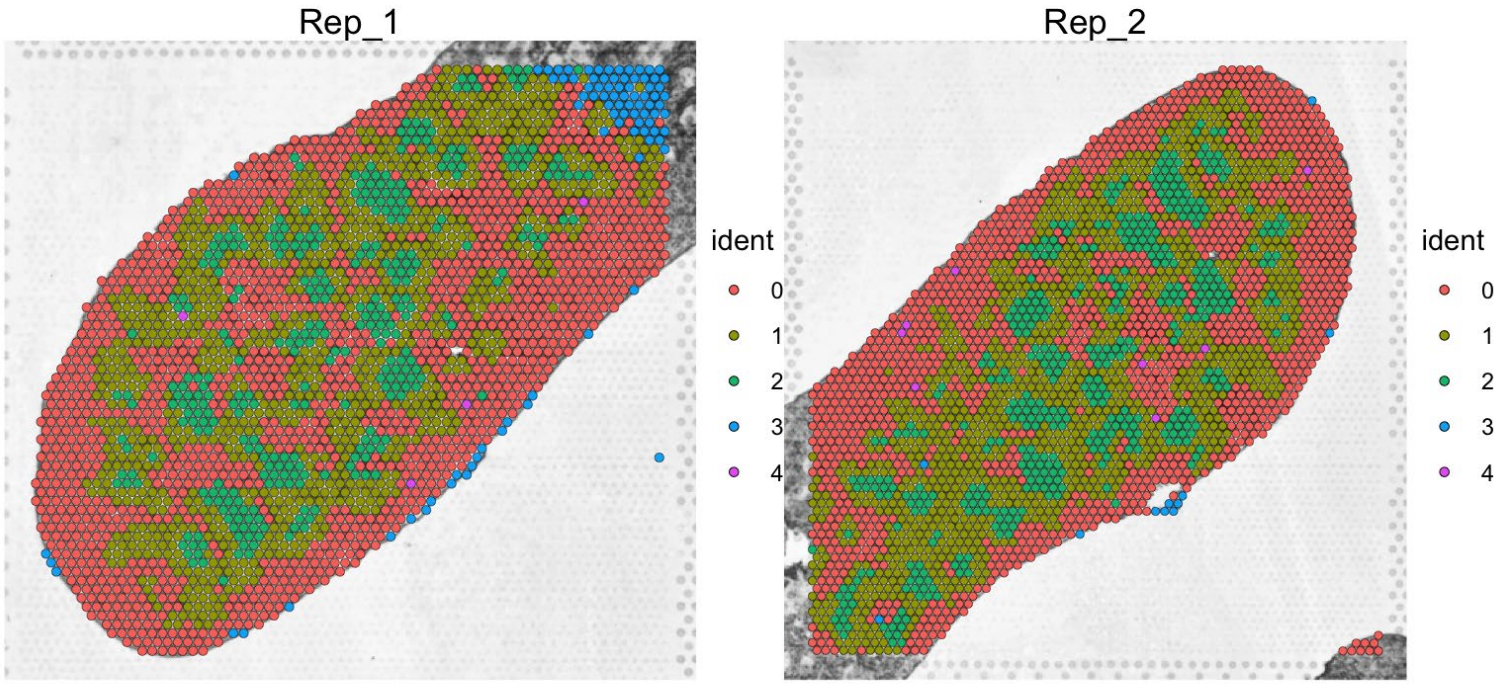
```
DefaultAssay(spleen) = "CITE"
spleen = NormalizeData(spleen, assay = "RNA", verbose = FALSE)
spleen = NormalizeData(spleen, normalization.method = "CLR", assay = "CITE", margin = 2, verbo
se = FALSE)
spleen <- ScaleData(spleen, verbose = FALSE)
p1 <- SpatialFeaturePlot(spleen,features = c("CD3","CD4","CD8",
                                             "CD19","B220-CD45R","IgD",
                                             "F4-80", "CD163", "CD68"), ncol = 3, images = c("
Rep_1"))
p2 <- SpatialFeaturePlot(spleen,features = c("CD3","CD4","CD8",
                                             "CD19","B220-CD45R","IgD",
                                             "F4-80", "CD163", "CD68"), ncol = 3, images = c("
Rep_2"))
p1 - p2
```



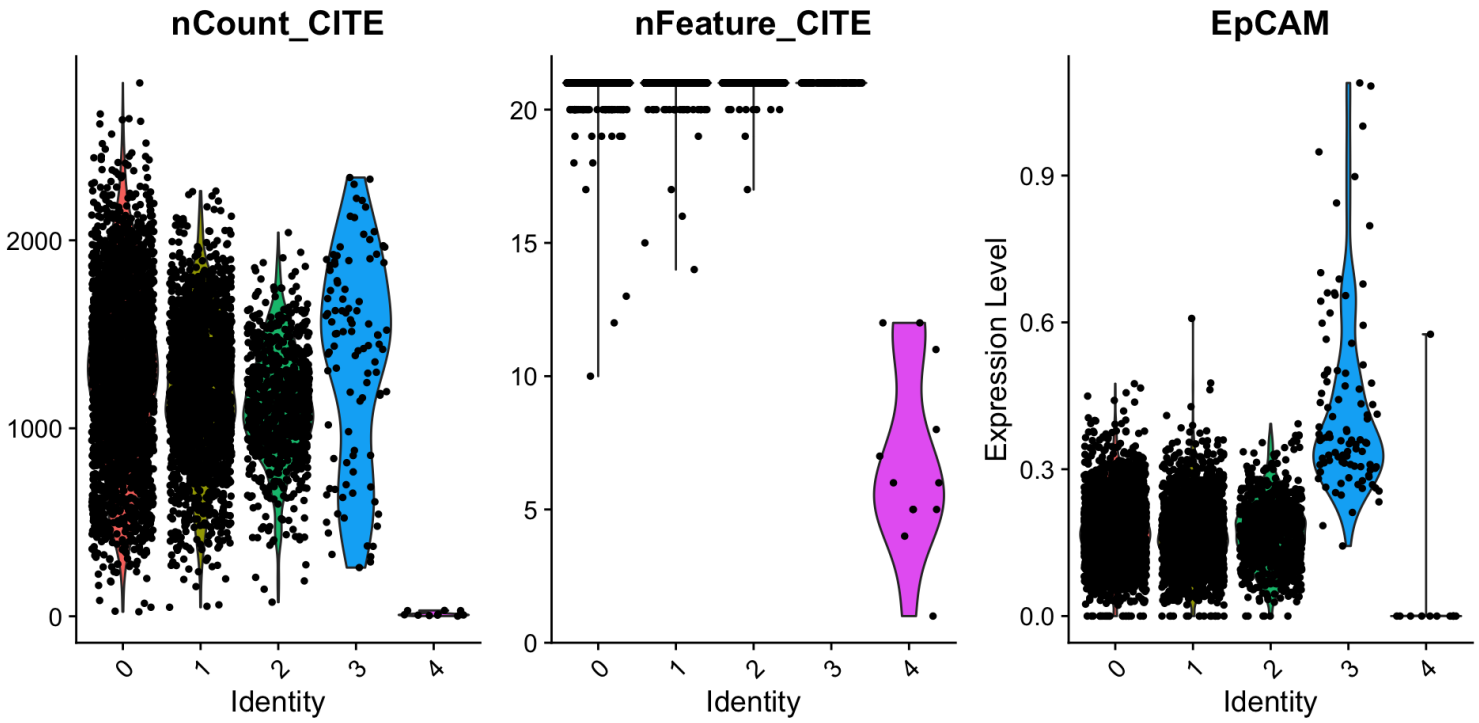


2. Dimensionality Reduction and Clustering

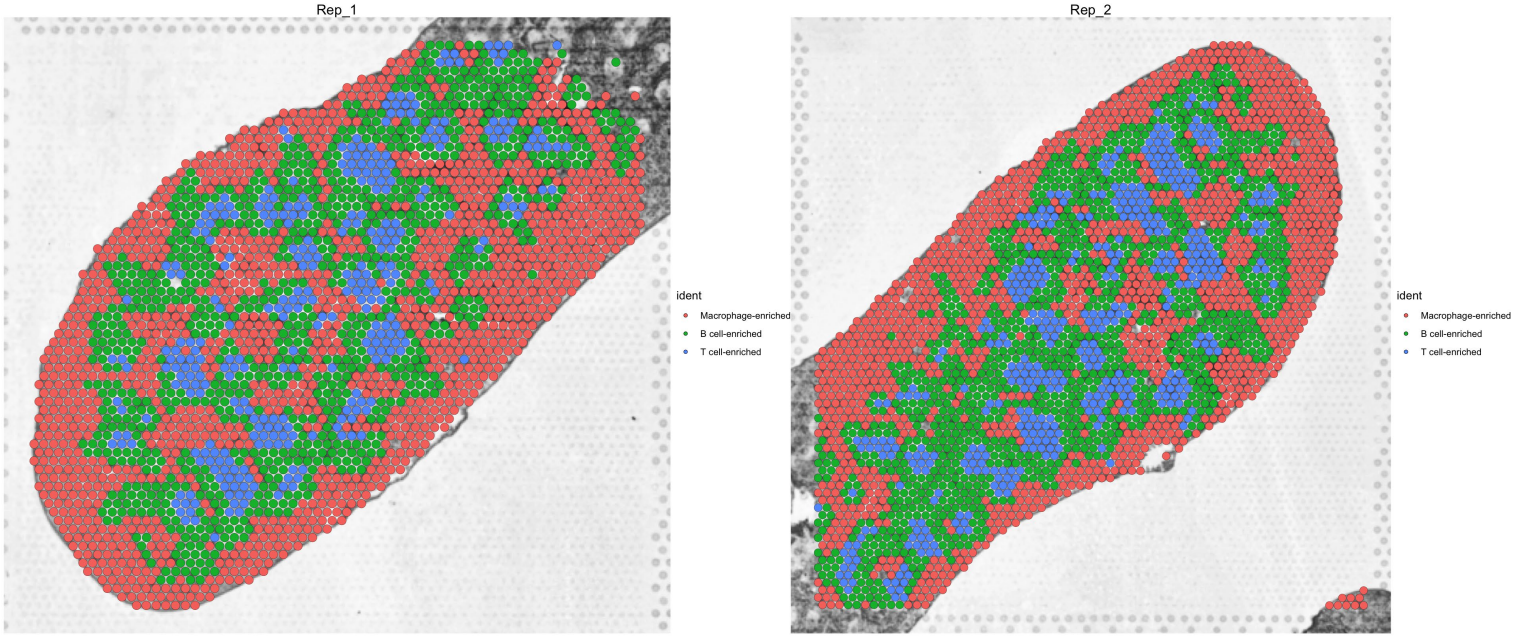
```
spleen <- RunPCA(spleen, features = rownames(spleen))
spleen <- FindNeighbors(spleen, dims = 1:10)
spleen <- FindClusters(spleen, resolution = 0.2, verbose = FALSE)
SpatialDimPlot(spleen, images = c("Rep_1","Rep_2"))
```




```
# Cluster 4 represents low-quality spatial barcodes
# Cluster 3 represents epithelial enriched tissue borders
VlnPlot(spleen, c("nCount_CITE", "nFeature_CITE", "EpCAM"))
```

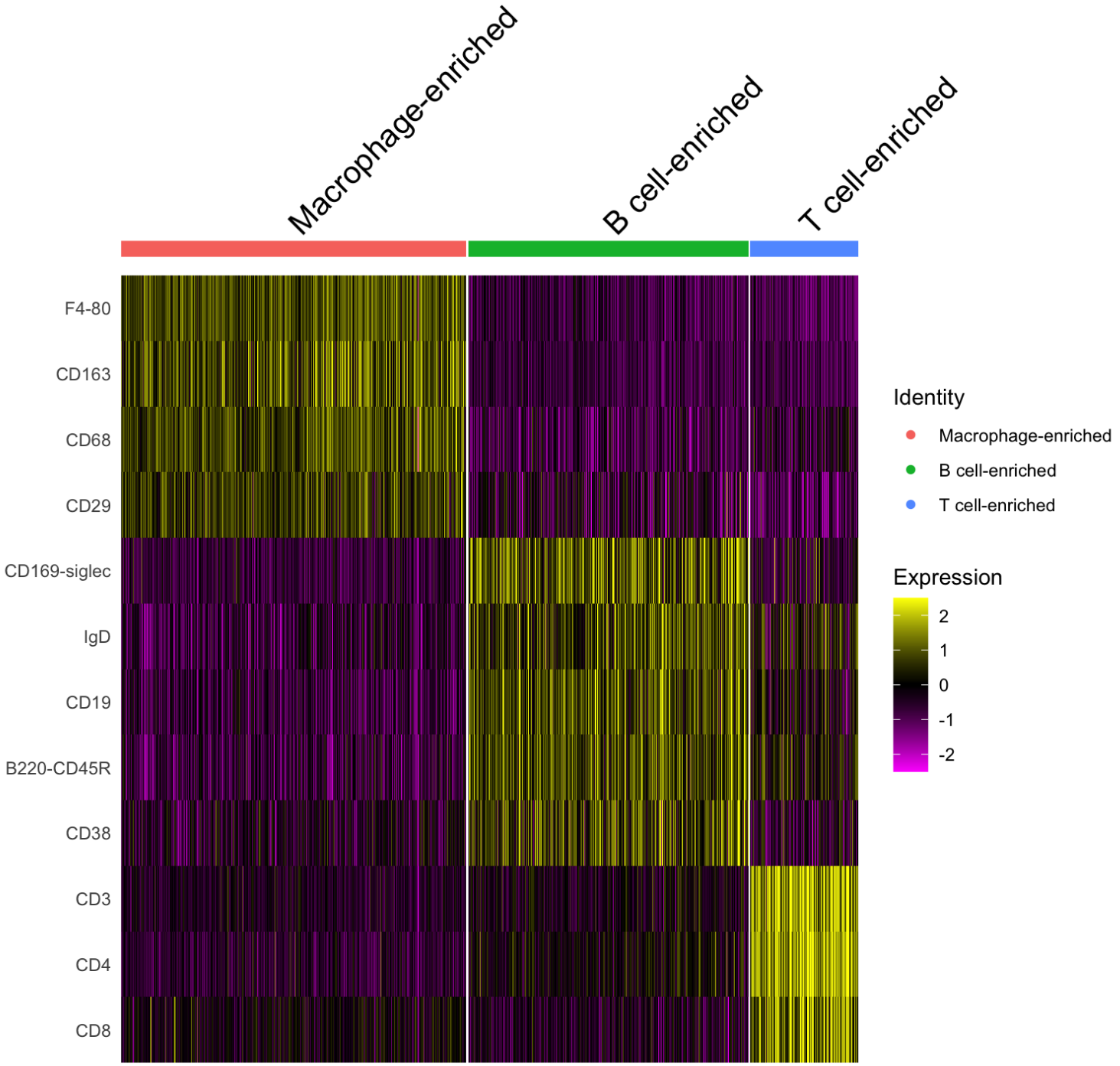



```
# rename clusters
spleen <- subset(spleen, subset = CITE_snn_res.0.2 %in% c(3,4), invert = TRUE)
spleen <- RenameIdents(spleen, '0' = "Macrophage-enriched", '1' = "B cell-enriched", '2' = "T cell-enriched")
SpatialDimPlot(spleen, images = c("Rep_1","Rep_2"))
```



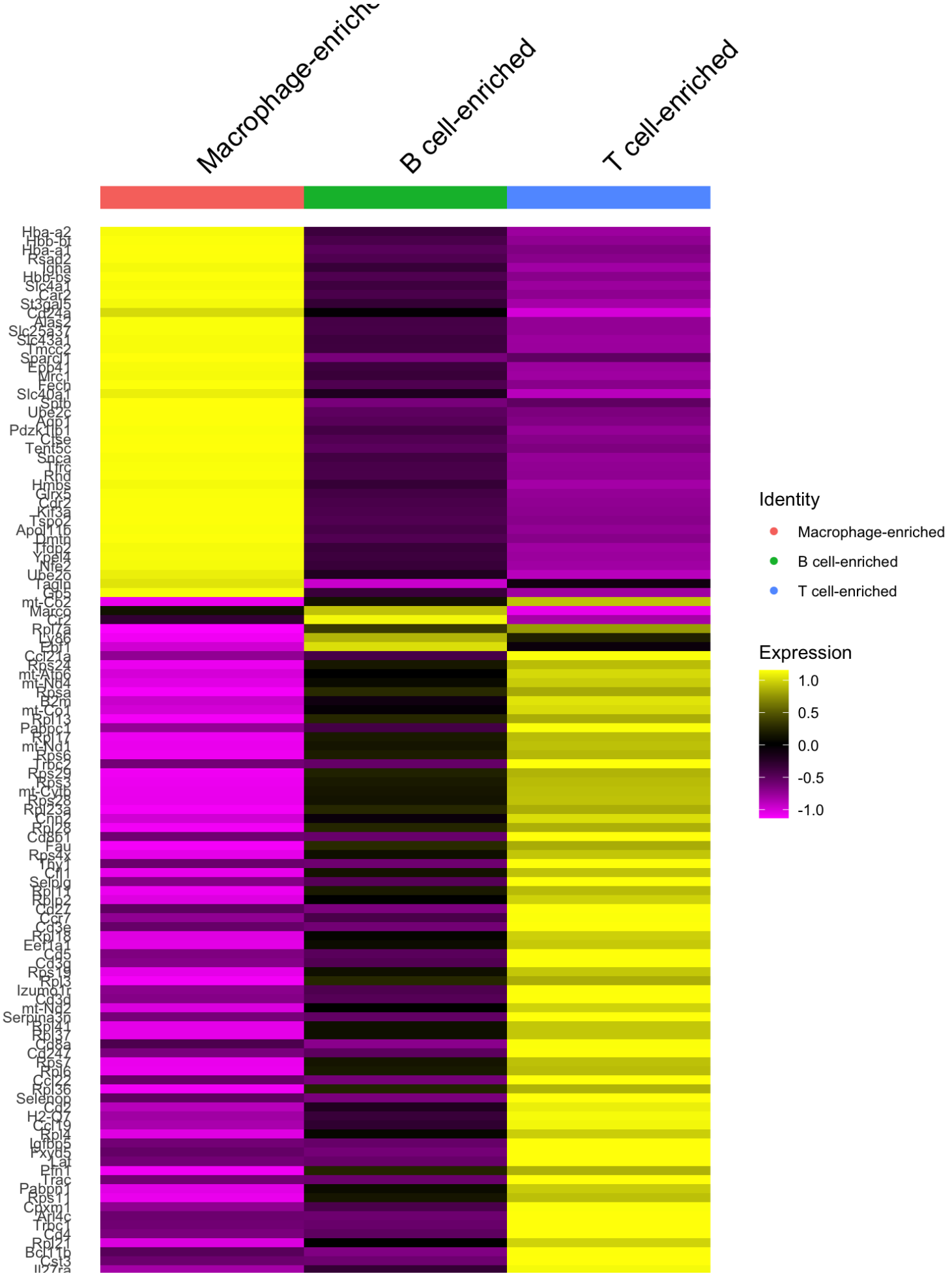
3. Differentially expressed ADTs

```
adt.markers <- FindAllMarkers(spleen, only.pos = TRUE, logfc.threshold = 0.2, verbose = FALSE)
DoHeatmap(spleen, adt.markers$gene, assay = "CITE", angle = 45)
```



4. Differentially expressed mRNAs

```
DefaultAssay(spleen) = "RNA"
mrna.markers <- FindAllMarkers(spleen, only.pos = TRUE, verbose = FALSE)
spleen.avg <- AverageExpression(spleen, assays = "RNA", return.seurat = TRUE)
DoHeatmap(spleen.avg, mrna.markers$gene, draw.lines = FALSE, assay = "RNA", angle = 45)
```


Macrophage-enriche
B cell-enriched
T cell-enriched
Identity
Macrophage-enriched
B cell-enriched
T cell-enriched
Expression
1.0
0.5
0.0
-0.5
-1.0

5. Session info

```
# Session info
print(sessionInfo())
```

```
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur 10.16
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRblas.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] SeuratObject_4.0.0 Seurat_4.0.1
##
## loaded via a namespace (and not attached):
##   [1] nlme_3.1-151          spatstat.sparse_2.0-0 matrixStats_0.57.0    bit64_4.0.5
   RcppAnnoy_0.0.18      RColorBrewer_1.1-2
##   [7] httr_1.4.2            sctransform_0.3.2     tools_4.0.3           R6_2.5.0
   irlba_2.3.3           rpart_4.1-15
##  [13] KernSmooth_2.23-18    uwot_0.1.10           mgcv_1.8-33           DBI_1.1.0
   lazyeval_0.2.2        colorspace_2.0-0
##  [19] withr_2.4.3           tidyselect_1.1.0      gridExtra_2.3         bit_4.0.4
   compiler_4.0.3        hdf5r_1.3.5
##  [25] plotly_4.9.2.2        labeling_0.4.2        scales_1.1.1          lmtest_0.9-38
   spatstat.data_1.7-0   ggridges_0.5.3
##  [31] pbapply_1.4-3         goftest_1.2-2         stringr_1.4.0         digest_0.6.27
   spatstat.utils_2.1-0  rmarkdown_2.6
##  [37] pkgconfig_2.0.3       htmltools_0.5.2       parallelly_1.23.0     limma_3.46.0
   fastmap_1.1.0         htmlwidgets_1.5.3
##  [43] rlang_0.4.10          shiny_1.5.0           farver_2.0.3          generics_0.1.0
   zoo_1.8-8             jsonlite_1.7.2
##  [49] ica_1.0-2             dplyr_1.0.2           magrittr_2.0.1        patchwork_1.1.1
   Matrix_1.3-2          Rcpp_1.0.7
##  [55] munsell_0.5.0         abind_1.4-5           reticulate_1.18       lifecycle_0.2.0
   stringi_1.5.3         yaml_2.2.1
##  [61] MASS_7.3-53           Rtsne_0.15            plyr_1.8.6            grid_4.0.3
   parallel_4.0.3        listenv_0.8.0
##  [67] promises_1.1.1        ggrepel_0.9.0         crayon_1.3.4          deldir_0.2-3
   miniUI_0.1.1.1        lattice_0.20-41
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##  [73] cowplot_1.1.1         splines_4.0.3         tensor_1.5            knitr_1.30
   pillar_1.4.7          igraph_1.2.6
##  [79] spatstat.geom_1.65-5  future.apply_1.7.0    reshape2_1.4.4        codetools_0.2-18
   leiden_0.3.6          glue_1.4.2
##  [85] evaluate_0.14         data.table_1.13.6     vctrs_0.3.6           png_0.1-7
   httpuv_1.5.4          polyclip_1.10-0
##  [91] gtable_0.3.0          RANN_2.6.1            purrr_0.3.4           spatstat.core_1.65-
5  tidyr_1.1.2           scattermore_0.7
##  [97] future_1.21.0         ggplot2_3.3.3         xfun_0.20             mime_0.9
   xtable_1.8-4          later_1.1.0.1
## [103] survival_3.2-7        viridisLite_0.3.0     tibble_3.0.4          cluster_2.1.0
   globals_0.14.0        fitdistrplus_1.1-3
## [109] ellipsis_0.3.1        ROCR_1.0-11
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