A customizable murine spectral backbone panel for immune surveillance in complex tissues

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Downtown NYC

Central Park

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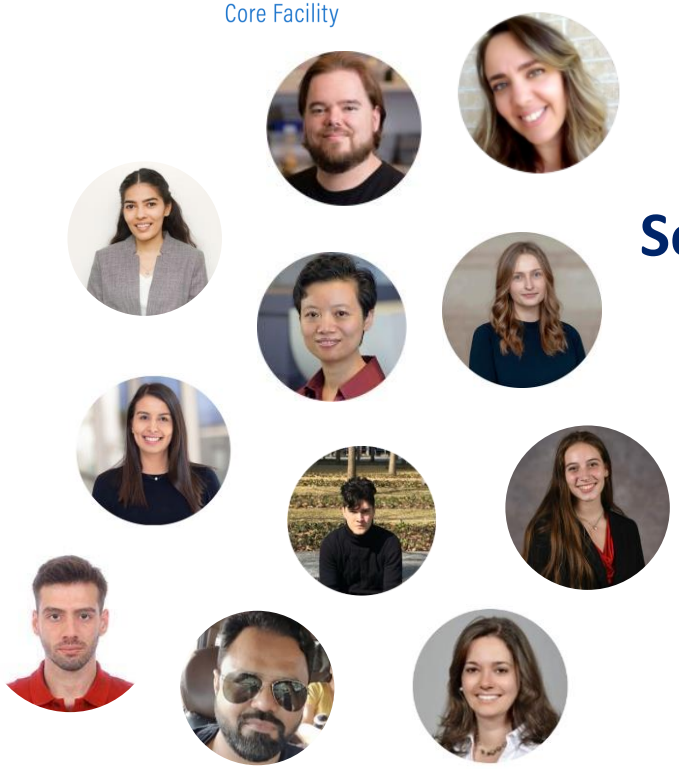

The FCCF Team

Sorters (10)

Spectral

#1 FACSDiscover S8
50+ colors

#2 FACSymphony S6
25+ colors

Polychromatic

#5 FACSAria
Up-to 18 colors

#2 SH800
Up-to 6 colors

Analyzers (7)

Spectral

#2 Aurora
40+ colors

#1 ID7000
40+ colors

Polychromatic

#3 Fortessa/LSRII
Up-to 18 colors

#1 CytoFLEX LX
Up-to 21 colors

~250 labs
~350 assisted sorts/month
~25 panels/year (6-25 markers)

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Now Offering:

PANEL DESIGN SERVICE

▪Company Agnostic

Panels are designed for any of our instruments with the best quality in mind considering all possible reagents, regardless of the vendor

▪Latest Knowledge of Biological Markers

With our knowledge in immunology and cancer biology we consider the most up-to-date markers that identify the immune populations of interest

▪FCCF staff available throughout

Staff are involved at every step and can more easily assist with troubleshooting

▪Support with data analysis

Assitance with FlowJo, FCSExpress, and OMIQ and high-dimensional analysis

SCAN ME

Panels for murine immune cells

Panel 1	Panel 2	Panel 3	Panel 4	Panel 5
CD8	CD45	CD45	CD127	CD3
CD4	CD4	CD3	CD135	CD4
MHC-II	CD3	CD4	Sca-1	CD8
Ly6G	CD8	CD8a	NK1.1	CD45
CD86	N1.1	NK1.1	CD3	CD11c
CD11c	CD25	CD11b	B220	MHC Class II
XCR1	CD11b	I-A/I-E	CD4	IDO-1
FOXP3	B220	CD86	CD8	Fox-P3
L/D	CD127	PDL1	CXCR3	CD123
Ki-67	Ly6G	ICOS	CCR4	Ly6C
CD45	CD44	PD-1	CD25	Ly6G
CD11b	CD69	FoxP3	CD11b	CD11b
CD103	CD62L	GranzymeB	Ly6C	B220
PD1	Ly6C	F4/80	F4/80	F4/80
TCRb		LY6C	MHC II	CD25
F4/80		Ly6G	Ly6G	
CD206		CD11c	CXCR4	
NKp46		B220	CD11c	
GzmB			CD127	
Ly6C				

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A 33-color panel of phenotypic analysis of murine organ specific immune cells

Si-Yu Yang [a,1], Meng-Xing Huang [a,1], Yan-Xia Sun [b,1], Liang Li [c], Zhen-Hua Bian [a], Jie Long [d], Zhi-Bin Zhao [c,*]

CANCER IMMUNOLOGY RESEARCH | RESEARCH ARTICLE

Longitudinal Immune Profiling Reveals Unique Myeloid and T-cell Phenotypes Associated with Spontaneous Immunoediting in a Prostate Tumor Model

Casey R. Ager[1], Aleksandar Z. Obradovic[1,2], Juan M. Arriaga[3], Matthew G. Chaimowitz[1], Andrea Califano[2,4,5,6,7,8], Cory Abate-Shen[2,3,4,9,10], and Charles G. Drake[1,9,11]

Using Full-Spectrum Flow Cytometry to Phenotype Memory T and NKT Cell Subsets with Optimized Tissue-Specific Preparation Protocols

Kathryn Farrand,[1,5] Lauren E. Holz,[2,5] Laura Ferrer-Font,[1,3] Michael D. Wilson,[1] Mitch Ganley,[4] Jordan J. Minnell,[1] Ching-Wen Tang,[1] Gavin F. Painter,[4] William R. Heath,[2] Ian F. Hermans,[1,3,6] and Olivia K. Burn[1,6,7]

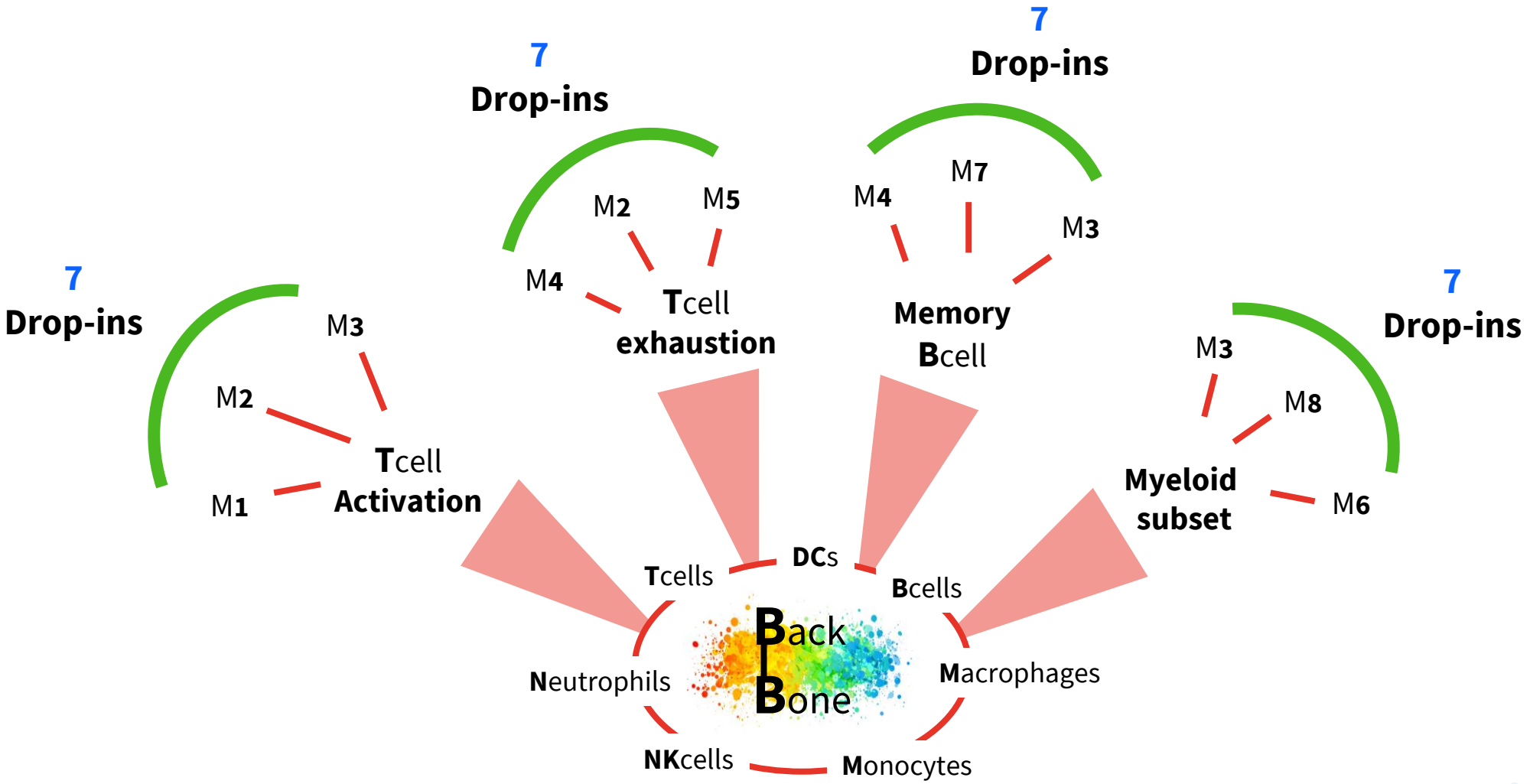
Cytometry PART A — Journal of Quantitative Cell Science — ISAC

OMIP | Open Access

OMIP-93: A 41-color high parameter panel to characterize various co-inhibitory molecules and their ligands in the lymphoid and myeloid compartment in mice

Johannes Brandi, Carsten Wiethe, Mathias Riehn, Thomas Jacobs

Immune surveillance of different subsets



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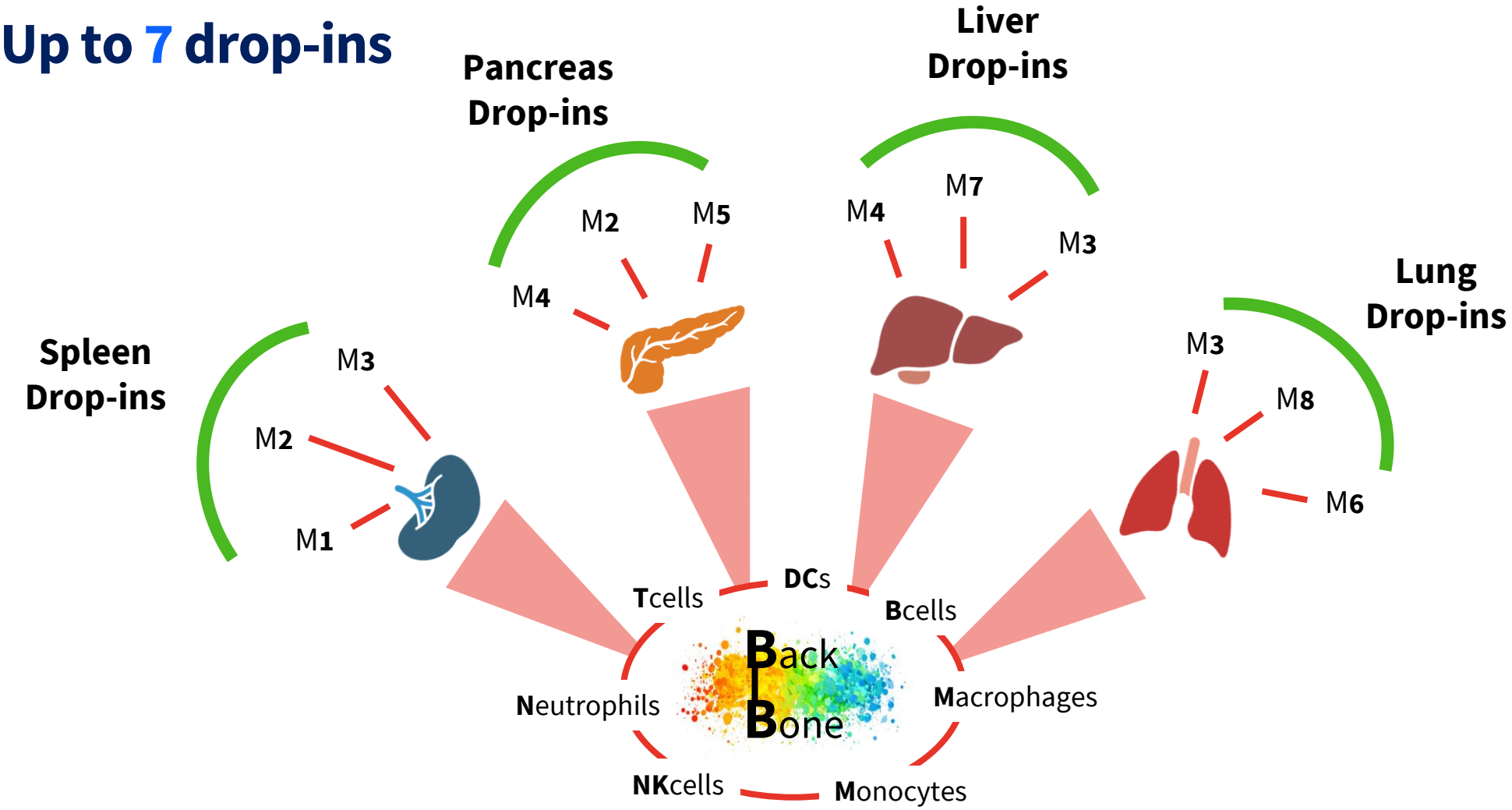
Immune surveillance of different tissues

Up to 7 drop-ins



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Murine spectral backbone panel for immune surveillance

frontiers | Frontiers in Immunology

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Ana Longhini Ines Maestre

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Development of a customizable mouse backbone spectral flow cytometry panel to delineate immune cell populations in normal and tumor tissues

Ana Leda F. Longhini[1*†], Inés Fernández-Maestre[2,3†], Margaret C. Kennedy[3,4], Matthew G. Wereski[2], Shoron Mowla[2], Wenbin Xiao[2,5,6], Scott W. Lowe[4,7], Ross L. Levine[2,5,8*†] and Rui Gardner[1*†]

[1]Flow Cytometry Core Facility, Memorial Sloan Kettering Cancer Center (MSKCC), New York, NY, United States, [2]Memorial Sloan Kettering Cancer Center, New York, NY, United States, [3]Louis V. Gerstner Jr Graduate School of Biomedical Sciences, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [4]Department of Cancer Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [5]Center for Hematologic Malignancies, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [6]Department of Pathology and Laboratory Medicine, Hematopathology Service, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [7]Howard Hughes Medical Institute, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [8]Department of Medicine, Leukemia Service, Memorial Sloan Kettering Cancer Center, New York, NY, United States

Introduction: *In vivo* studies of cancer biology and assessment of therapeutic

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Building a backbone panel

Purpose:

Mouse Immuno-profiling of tumor samples and other disease models

- Characterize the major immune populations
- Expandable and customizable panel – pre-defined drop-in fluorochromes
- Work for a variety of tissues
- Work on any spectral cytometer

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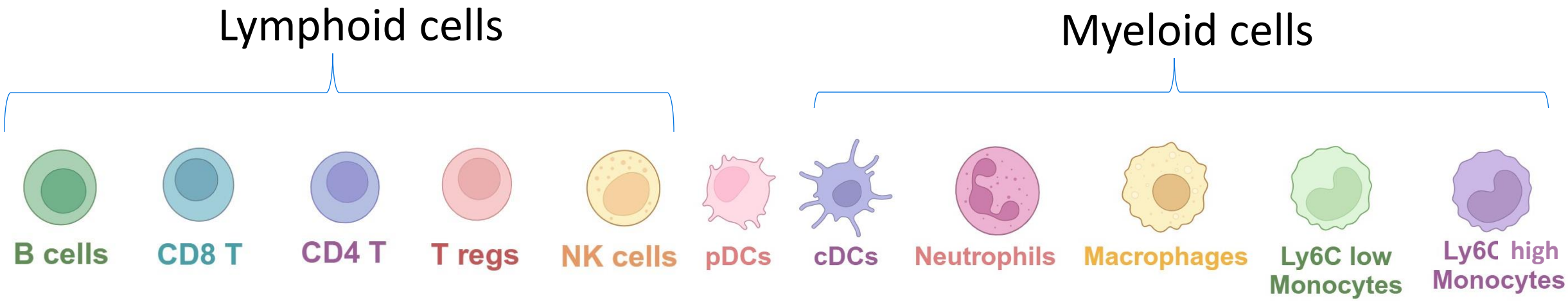
Populations

Immune populations



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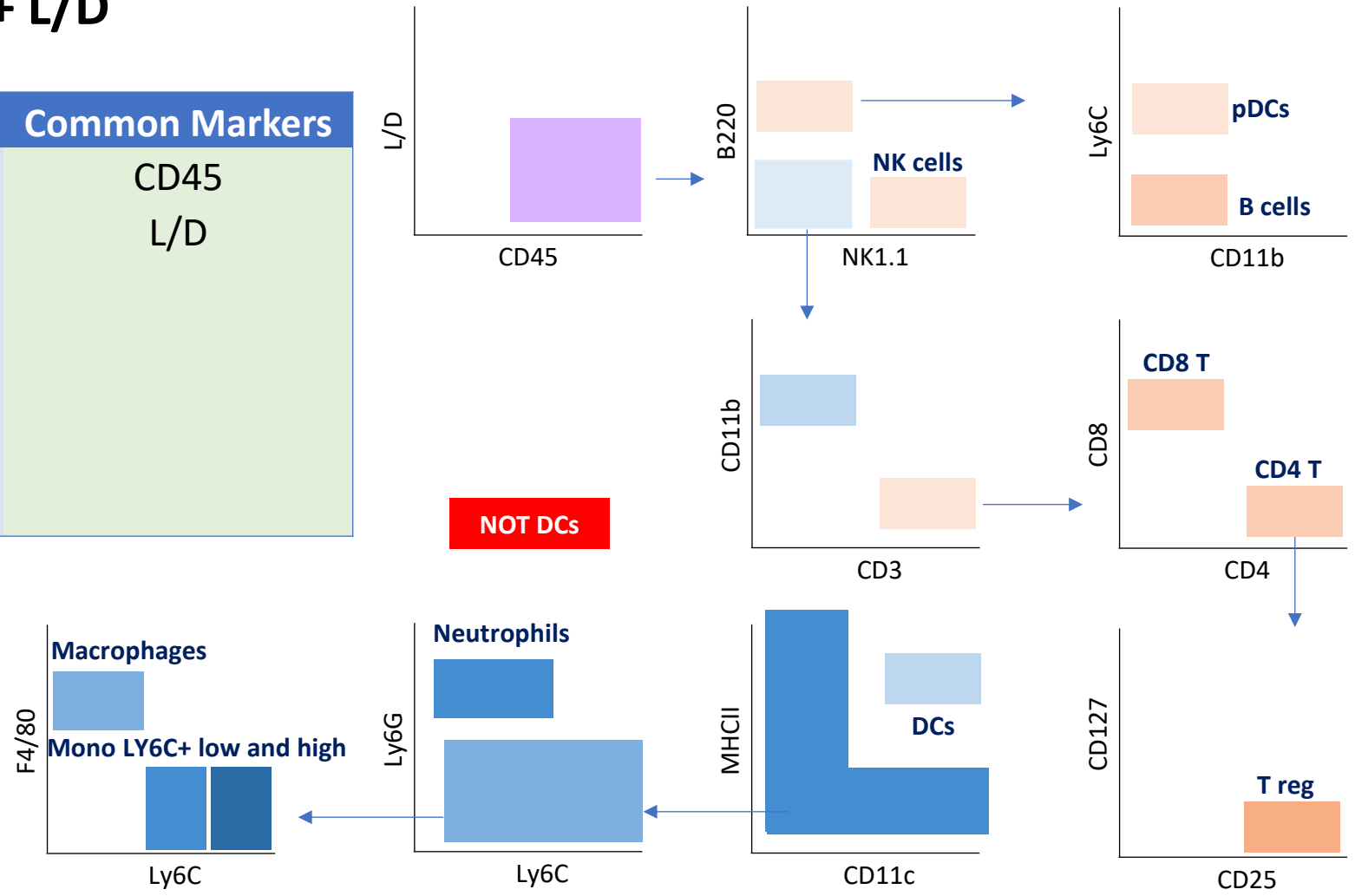
Backbone Markers and gating strategy

Markers

Backbone: 14 markers + L/D

Lymphoid Markers	Myeloid Markers	Common Markers
CD3	CD11b	CD45
B220	Ly6C	L/D
CD4	F4/80	
CD8	MHC II	
NK1.1	LY6G	
CD25	CD11c	
CD127		

Gating strategy allows definition of **11** subpopulations



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Drop-in fluors

Drop-ins – the logic behind

- Fluors: Bright and common
- Minimal impact on the Backbone resolution and vice-versa
- Minimal impact between each other

➢First-choice drop-ins : BV421, FITC (or BB515), PE, APC

Another valuable point: First-choice Drop-ins are not tandem dyes - less problems when unmixing – fluor library

➢Suggestions for additional drop-ins : BV605, BV786, PE-Cy7

The backbone fluor assignment

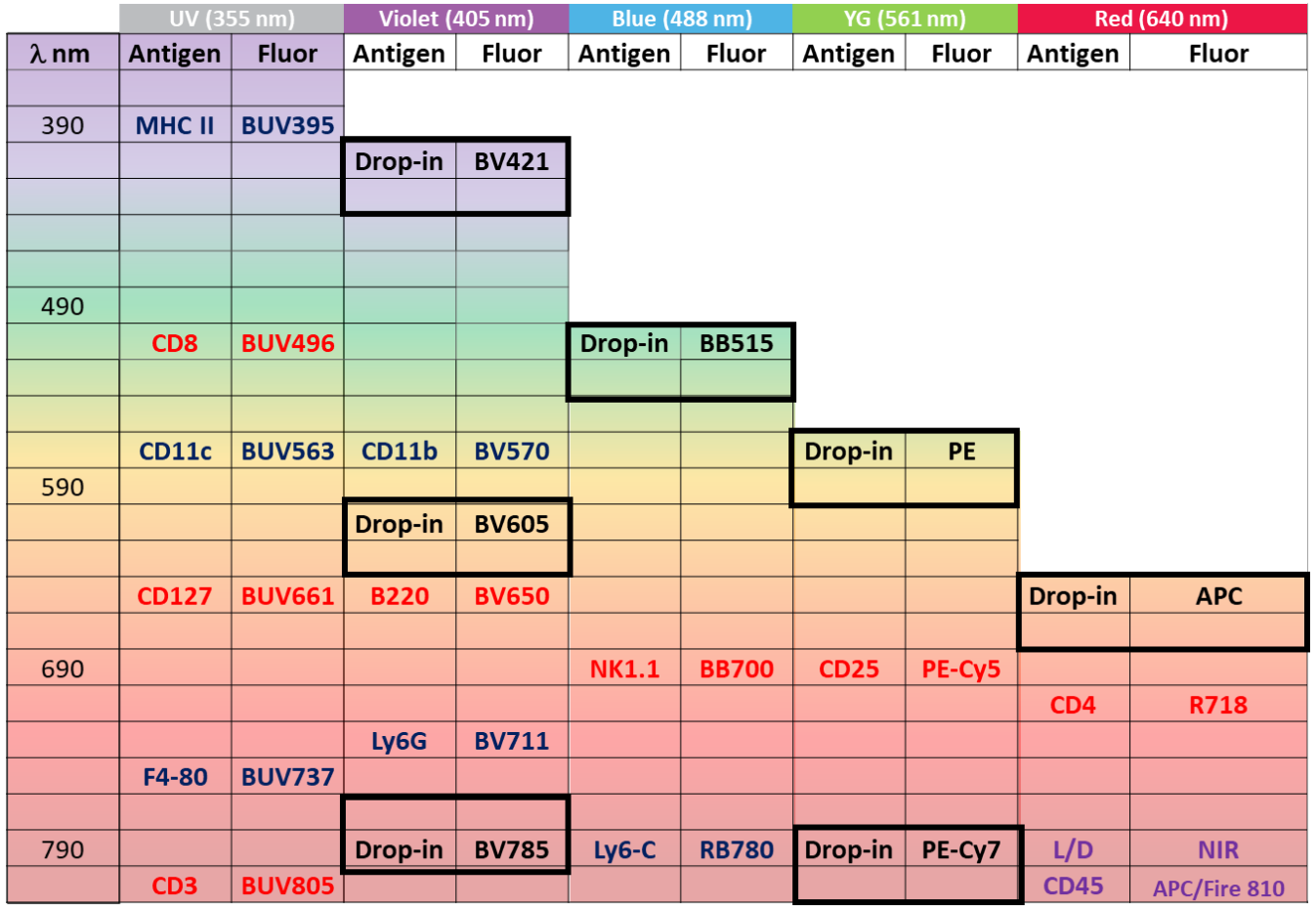
BackBone fluors

Respect the rules for panel design

1. Bright fluors – low expression and dim fluors – high expression
2. Spread: impact on co-expressed markers

Intercalate myeloid markers with lymphoid markers on the same laser line and avoid co-expression across lasers

Fluorochrome assignment



	UV (355 nm)		Violet (405 nm)		Blue (488 nm)		YG (561 nm)		Red (640 nm)	
λ nm	Antigen	Fluor	Antigen	Fluor	Antigen	Fluor	Antigen	Fluor	Antigen	Fluor
390	MHC II	BUV395								
			Drop-in	BV421						
490										
	CD8	BUV496			Drop-in	BB515				
	CD11c	BUV563	CD11b	BV570			Drop-in	PE		
590										
			Drop-in	BV605						
	CD127	BUV661	B220	BV650					Drop-in	APC
690					NK1.1	BB700	CD25	PE-Cy5		
									CD4	R718
			Ly6G	BV711						
	F4-80	BUV737								
790			Drop-in	BV785	Ly6-C	RB780	Drop-in	PE-Cy7	L/D	NIR
	CD3	BUV805							CD45	APC/Fire 810

- **Myeloid markers**
- **Lymphoid markers**
- **Common marker**
- **Drop-in fluorochromes**

Backbone **15 parameters** (14 surface markers + L/D)

+

Drop-ins **7 custom markers**

22 parameter panel

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Steps for panel evaluation

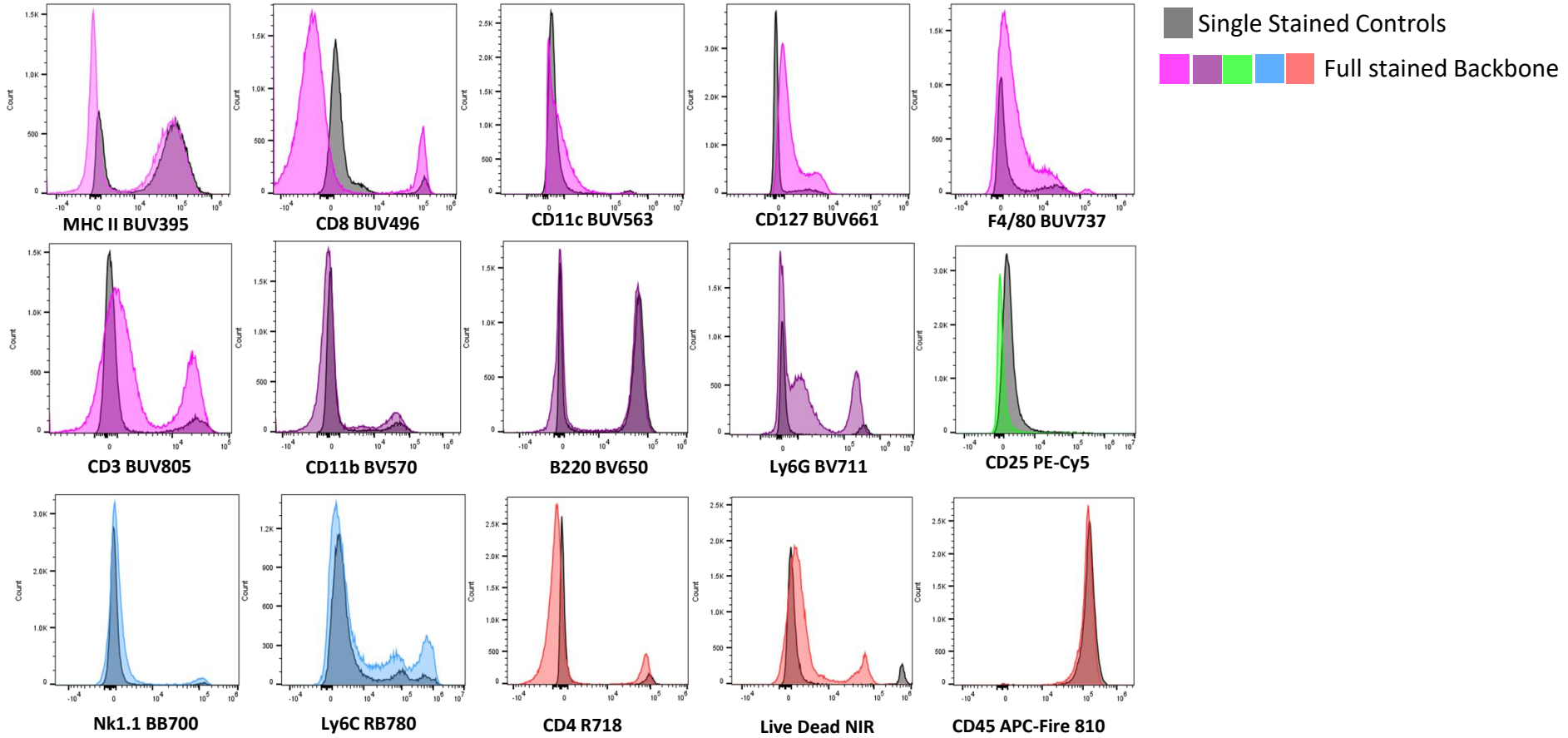
Panel evaluation

- The backbone panel clearly resolves major immune cells subsets
- The backbone panel must have **minimal** impact on the resolution of the drop-in fluorochromes
- The drop-in fluors must have **minimal** impact on the backbone resolution

Backbone panel N x N plots – unmixing evaluation

Comparison of single stained controls with fully stained backbone sample

Panel evaluation



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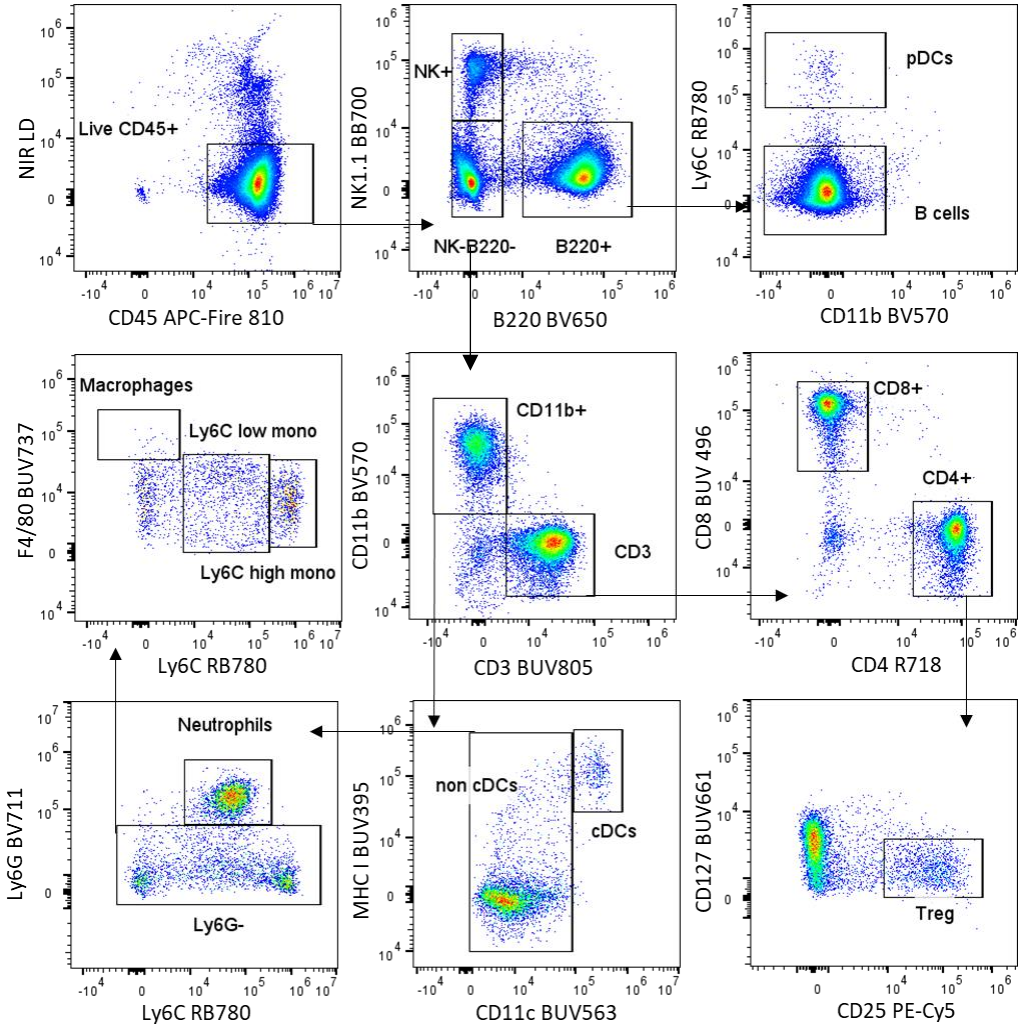
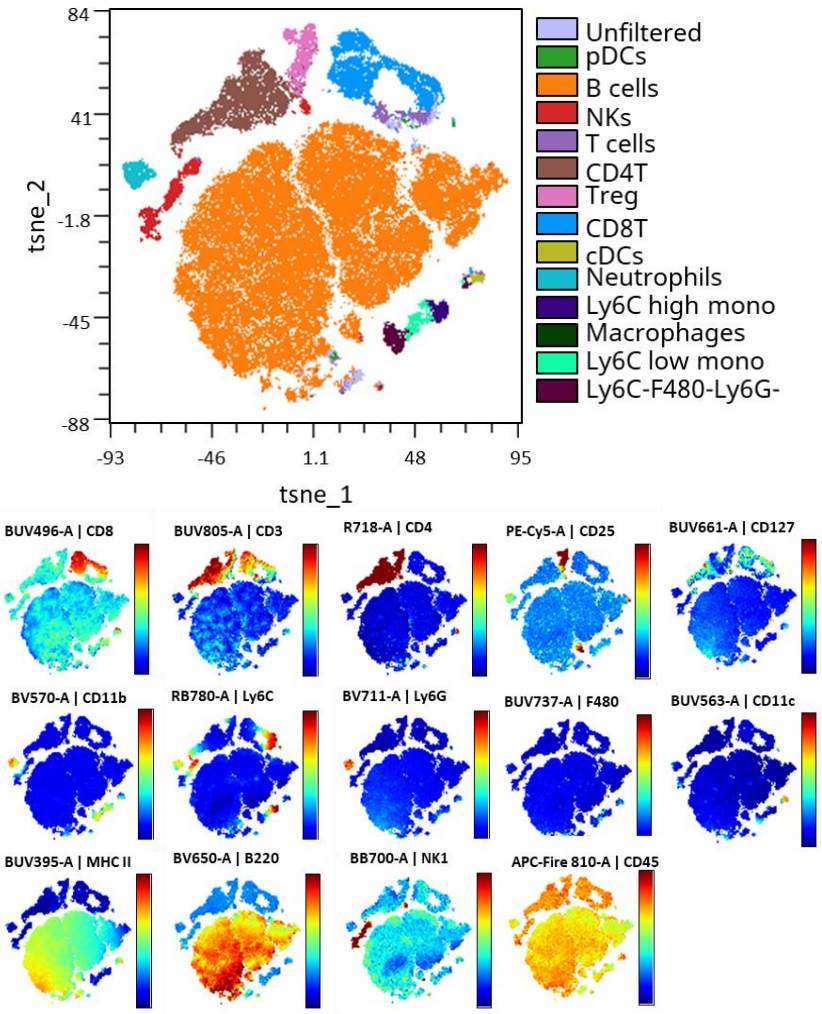
Backbone panel: manual and unsupervised analysis





Panel evaluation

Sample: spleen cells from C57B6/N mice

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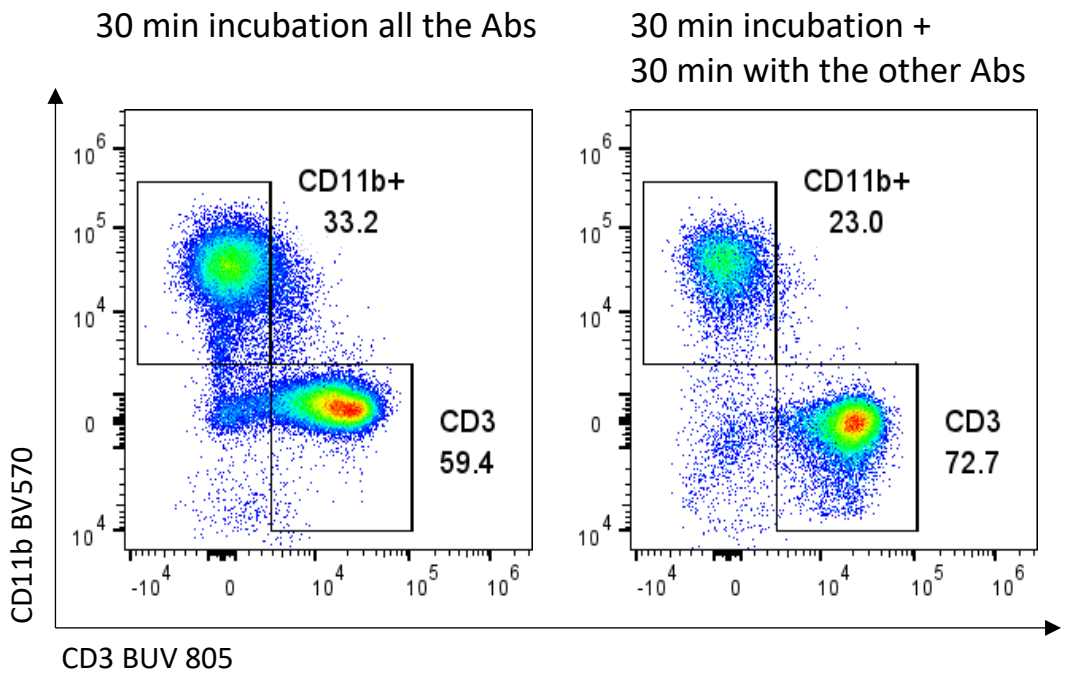
CD3 signal is improved by increasing the incubation time



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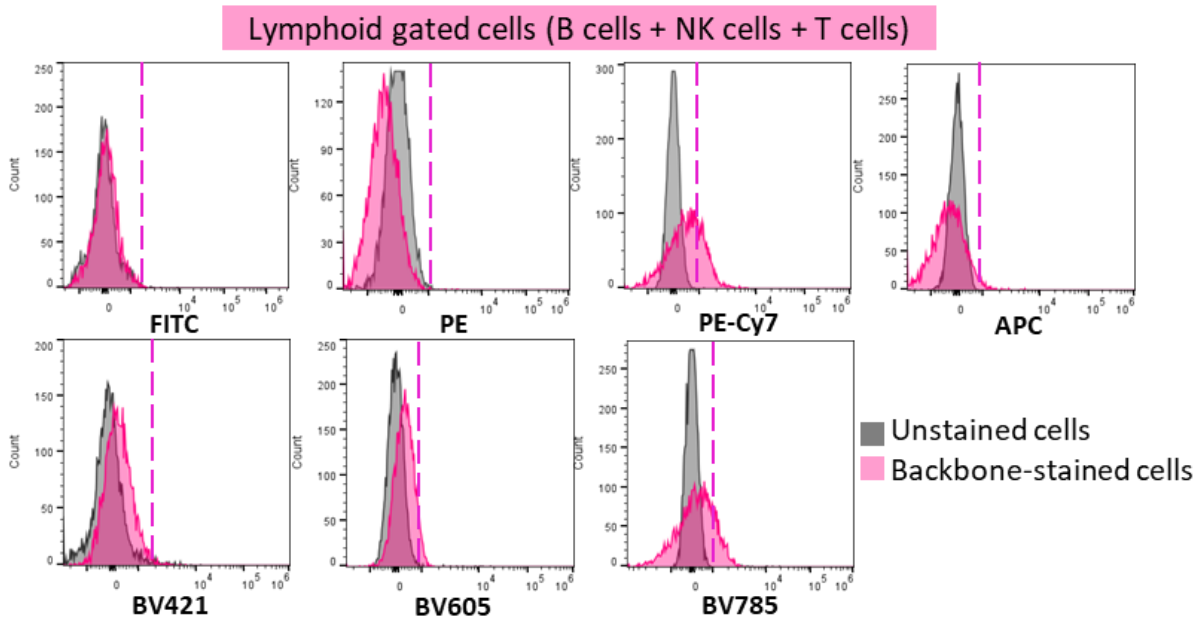
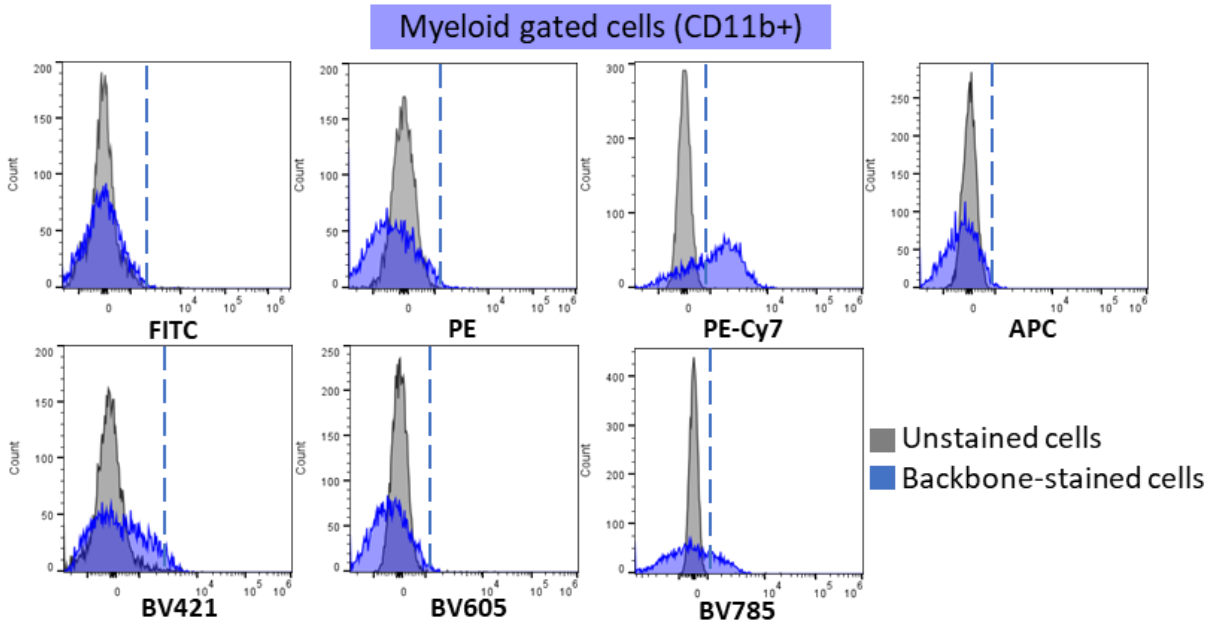
Impact of the backbone panel on the drop-in fluorochromes

Panel evaluation





Unstained and fully stained sample: C57B6/N mice splenocytes
Single stained controls for the drop-in fluorochromes: splenocytes were stained with CD4 for each individual fluorochrome

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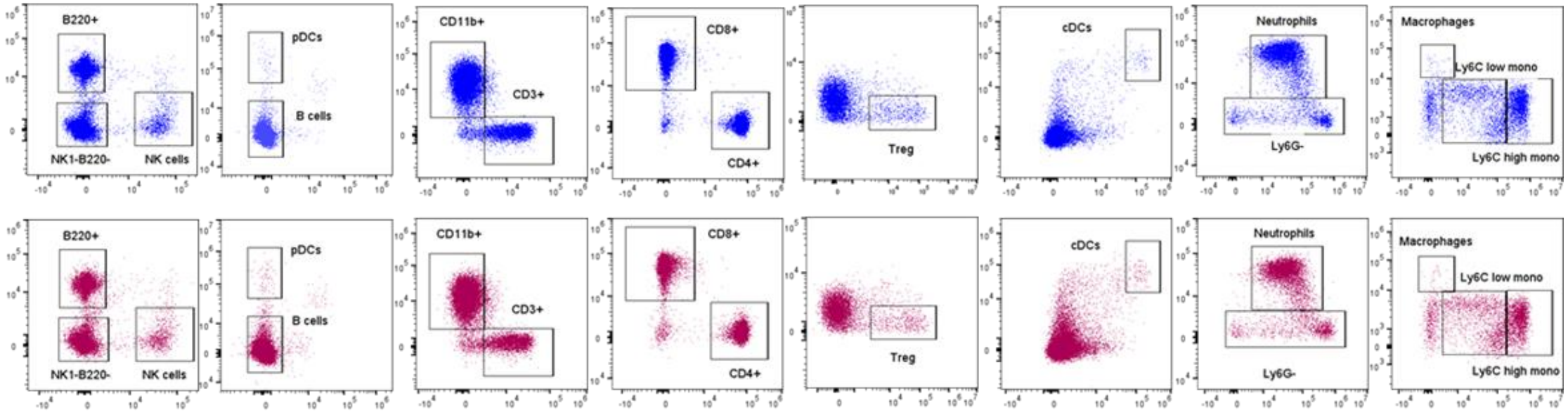
The addition of drop-ins does not impact the backbone resolution on spleen samples

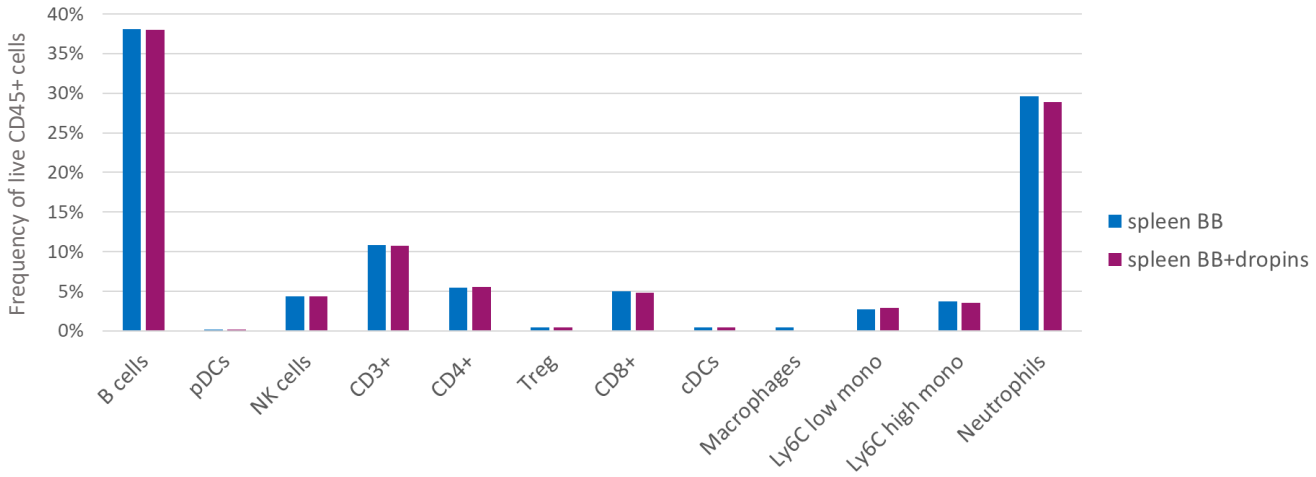
Spleen cells

Backbone only

Backbone + drop-ins Immune panel



Drop-ins Immune panel	
Fluor	**Marker**
BV421	CD62L
BV605	Siglec-F
BV785	c-Kit
BB515	------
FITC	CD44
PE	TIM-4
PE-Cy7	PD-1
APC	Lag-3



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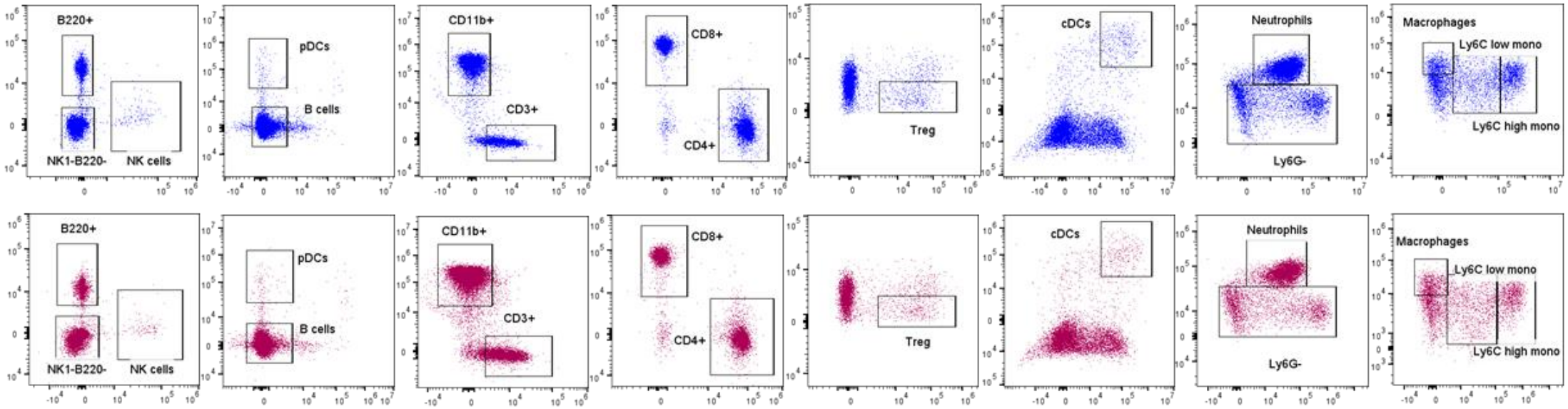
The addition of drop-ins does not impact the backbone resolution on KRAS-driven lung adenocarcinoma

Lung tumor

Backbone only

Backbone + drop-ins TME panel



Samples: dissociated lungs from C57Bl6/N mice injected with KrasG12C/+; Trp53fl/fl lung cells.

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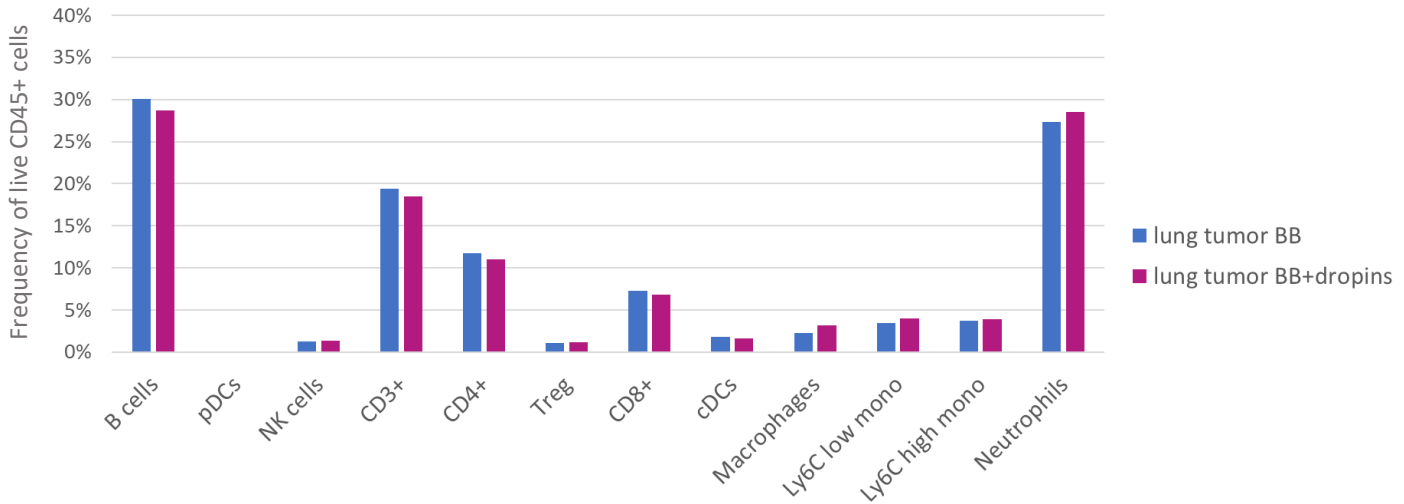
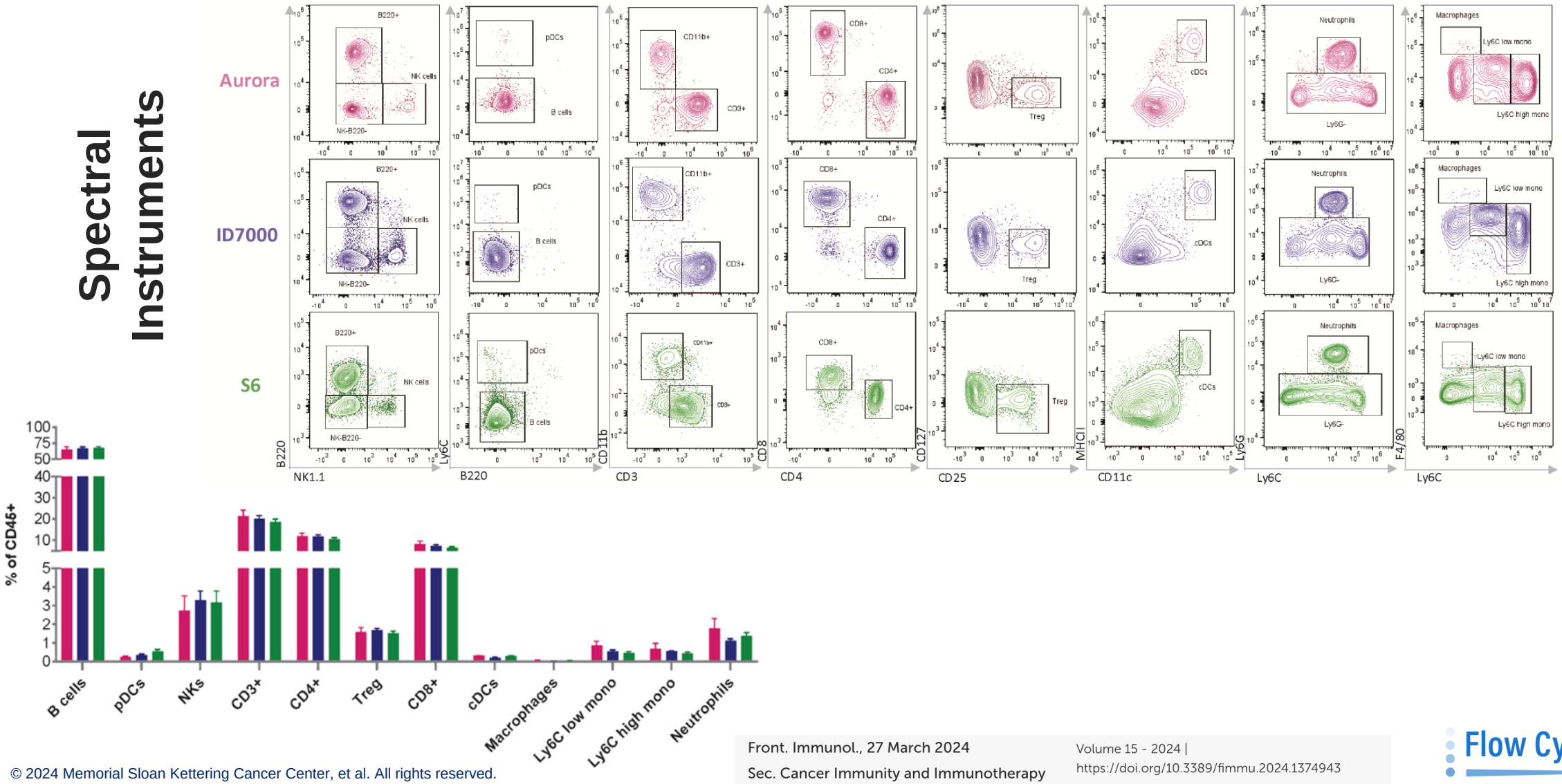
Drop-ins TME panel	
Fluor	**Marker**
BV421	PDPN
BV605	Epcam
BV785	------
BB515	Lag-3
FITC	------
PE	------
PE-Cy7	PD-1
APC	CD31



The backbone panel performs across different spectral cytometers



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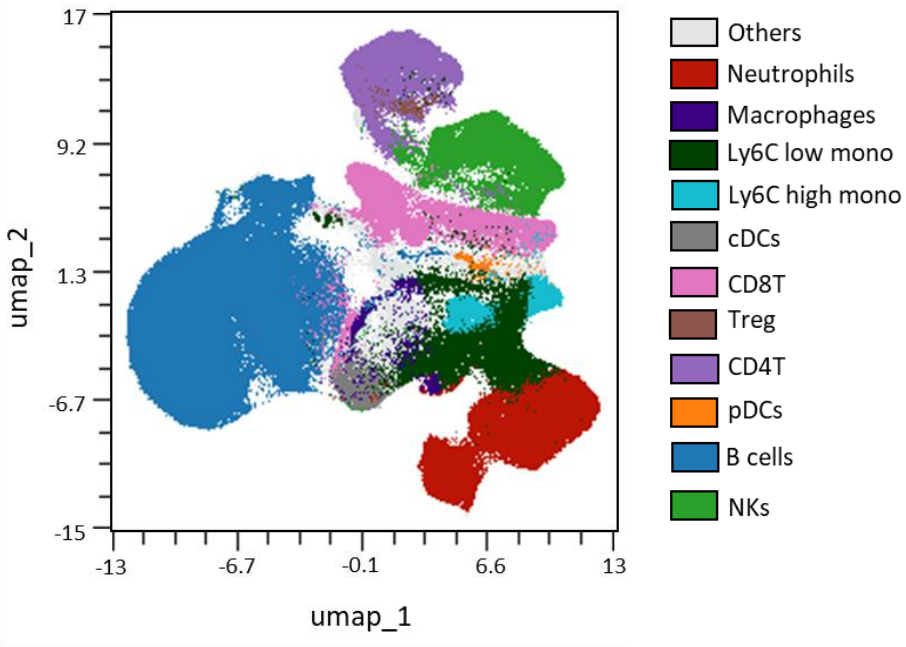
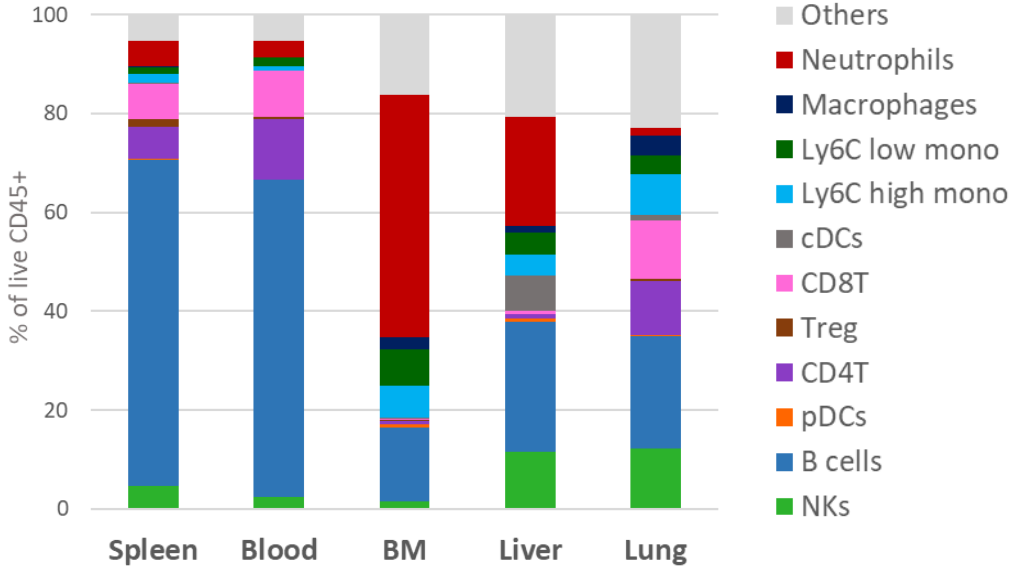
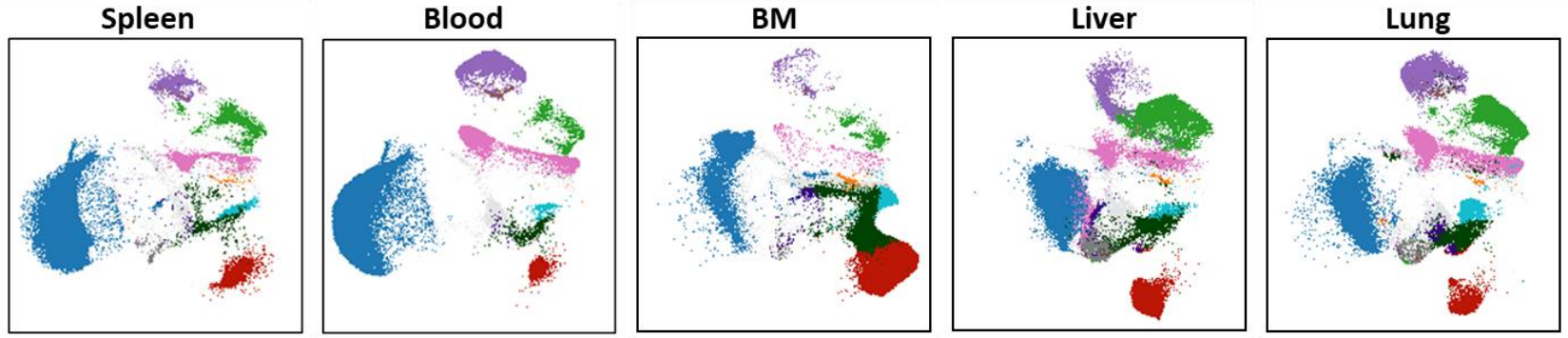
The backbone is organ agnostic







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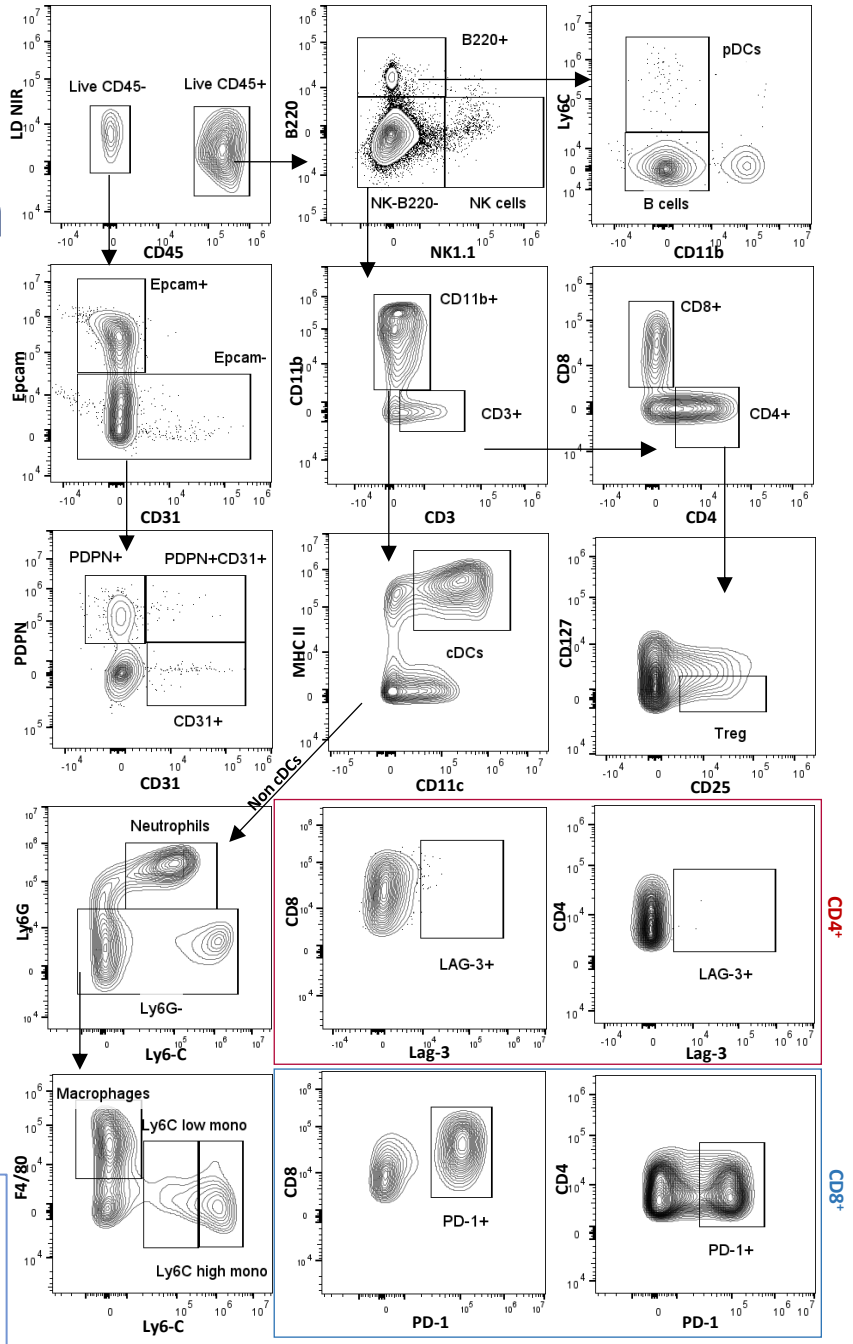
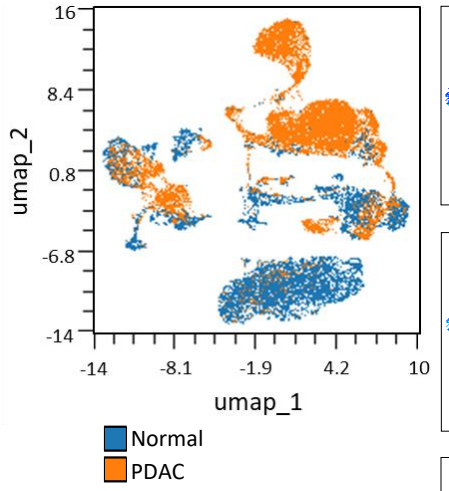
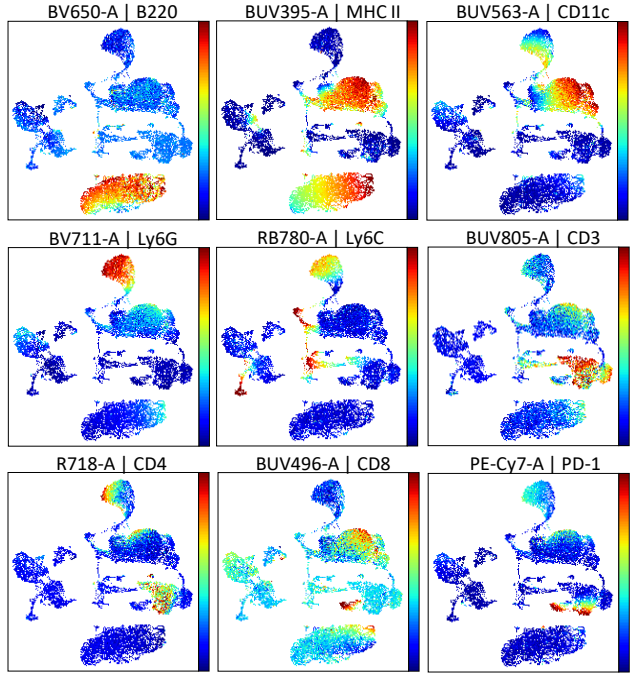
The backbone can be used with complex tumor samples

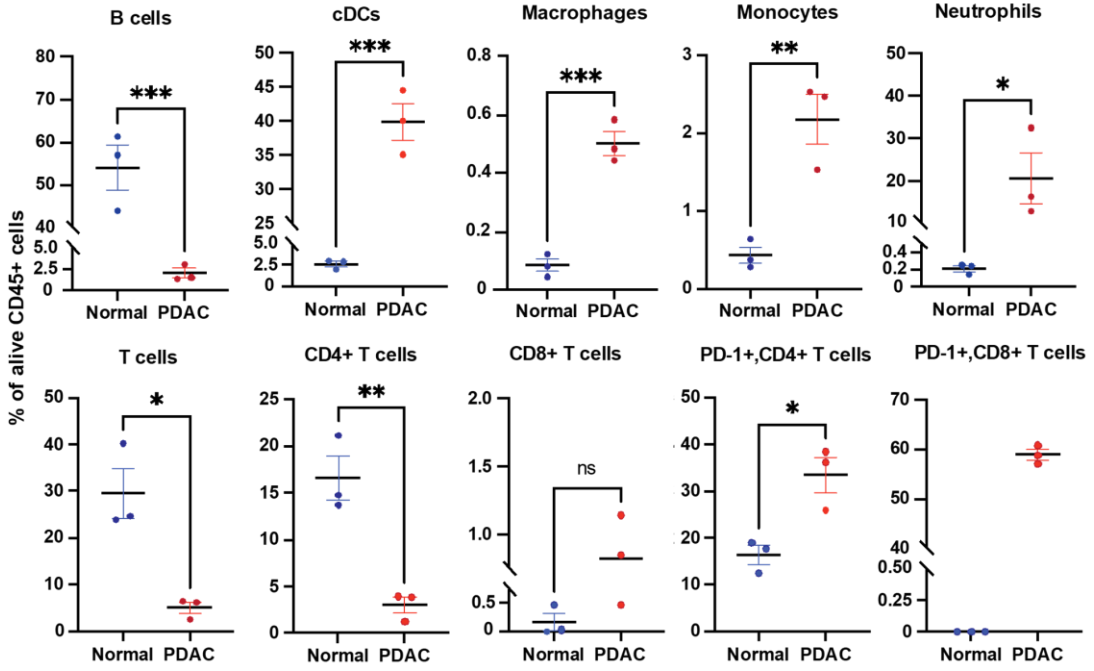
Drop-ins TME panel	
Fluor	**Marker**
BV421	PDPN
BV605	Epcam
BV785	------
BB515	Lag-3
FITC	------
PE	------
PE-Cy7	PD-1
APC	CD31

Samples: pancreatic ductal adenocarcinoma (PDAC) and normal dissociated pancreas from C57Bl6/N





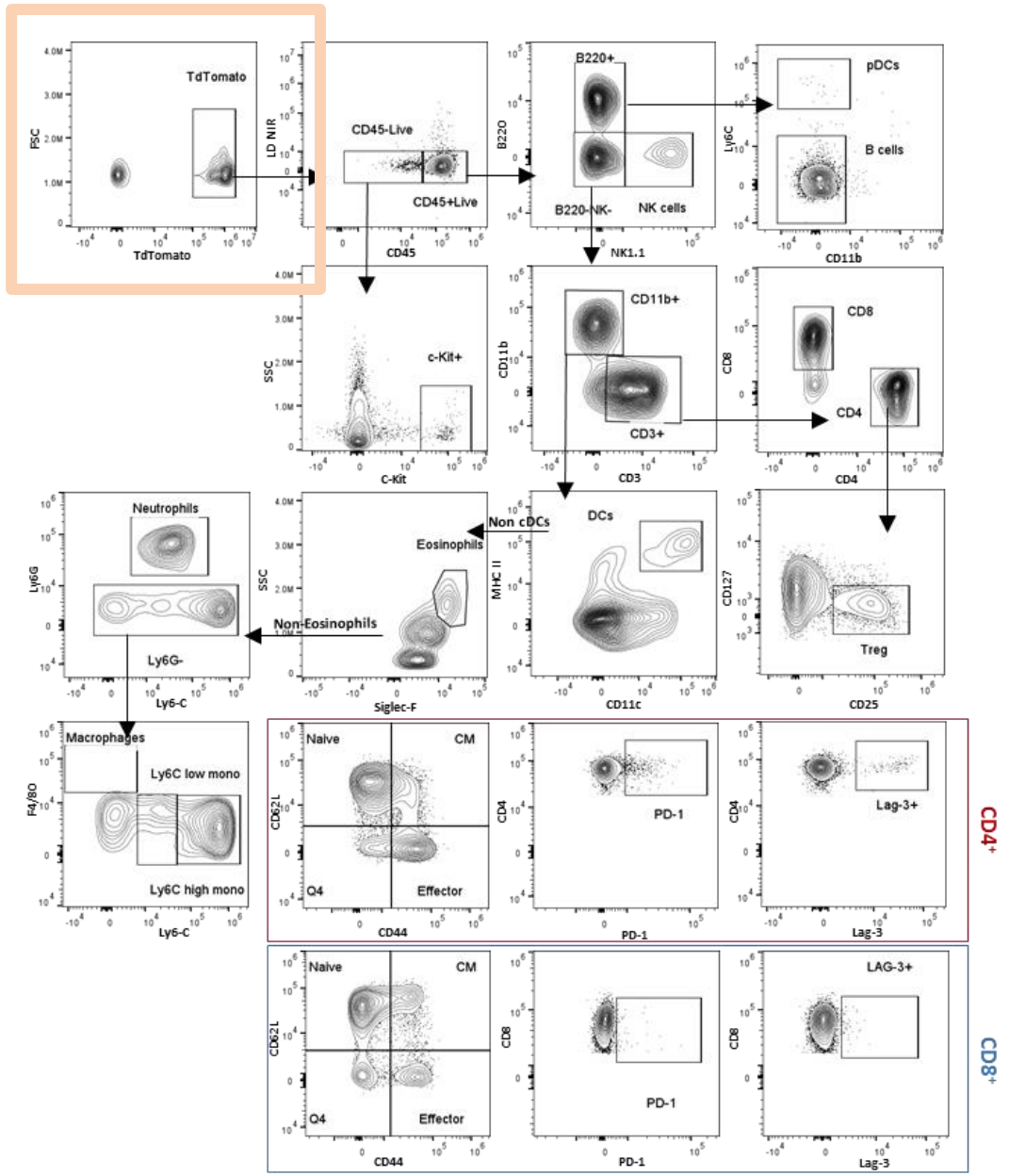




The backbone performs with highly expressed TdTomato cells

Drop-ins Immune panel TdTomato	
Fluor	**Marker**
BV421	CD62L
BV605	Siglec-F
BV785	c-Kit
BB515	------
FITC	CD44
PE	------
PE-Cy7	PD-1
APC	Lag-3
TdTomato	TdTomato

Sample: splenocytes from tdT+ HSC-Scl-Cre-ERT C57Bl6/N mouse



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Future improvements

- Improve CD3 resolution
  Swap fluor (BUV805)
  Alternatively use TCR$\alpha\beta$
- Develop backbone panel for intracellular staining
- Develop human backbone panel
- Explore autofluorescence extraction to improve resolution

Summary

- The backbone panel is reliable for profiling immune cells from hematopoietic and non-hematopoietic organs, as well as tumors with complex immune microenvironments.
- The backbone panel maintains its resolution across different spectral flow cytometers.
- The panel is validated to incorporate up to seven other fluorochromes and can be associated with bright fluorescent proteins, such as tdTomato.
- A robust backbone that can be customized with pre-tested drop-in fluorochromes not only saves time and resources, but also brings consistency and standardization, making it a valuable solution for immuno-oncology researchers.

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Diana Vesely (**Biolegend**)

Mark Edinger (**Cytek**)

Ana Longhini

(Scientific Manager)

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Memorial Sloan Kettering Cancer Center

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Building Flow Cytometry tools to improve research

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Questions?

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