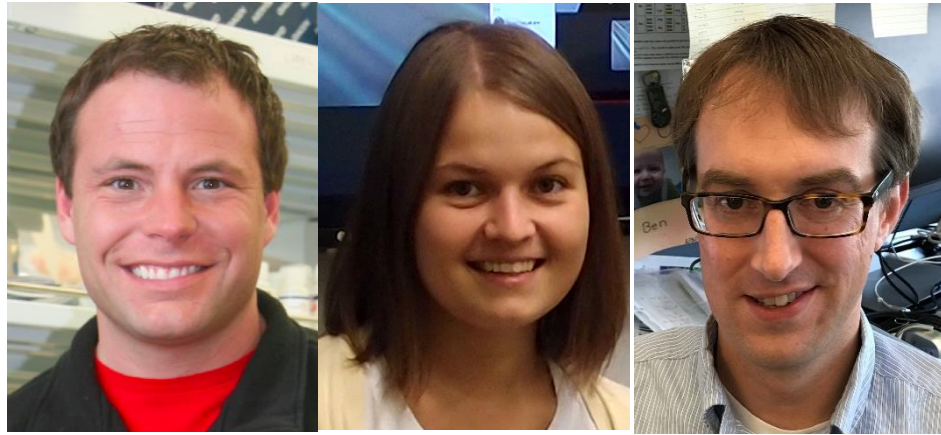


A 3D molecular model of a cell, showing various proteins and structures. The cell is rendered in a light, translucent style, with several blue and orange structures protruding from its surface. The background is a soft, out-of-focus light blue and white.

Single-cell dissection of the checkpoint immunotherapy

CANCER
IMMUNOTHERAPY

Max Artyomov
Sep 24, 2019



**Matt
Gubin**

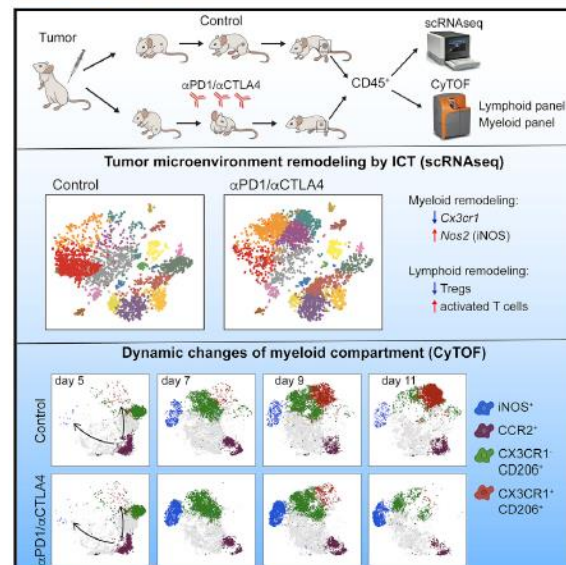
**Katya
Esaulova**

**Jeff
Ward**

Cell

High-Dimensional Analysis Delineates Myeloid and Lymphoid Compartment Remodeling during Successful Immune-Checkpoint Cancer Therapy

Graphical Abstract



Authors

Matthew M. Gubin, Ekaterina Esaulova, Jeffrey P. Ward, ..., Stephen T. Oh, Robert D. Schreiber, Maxim N. Artyomov

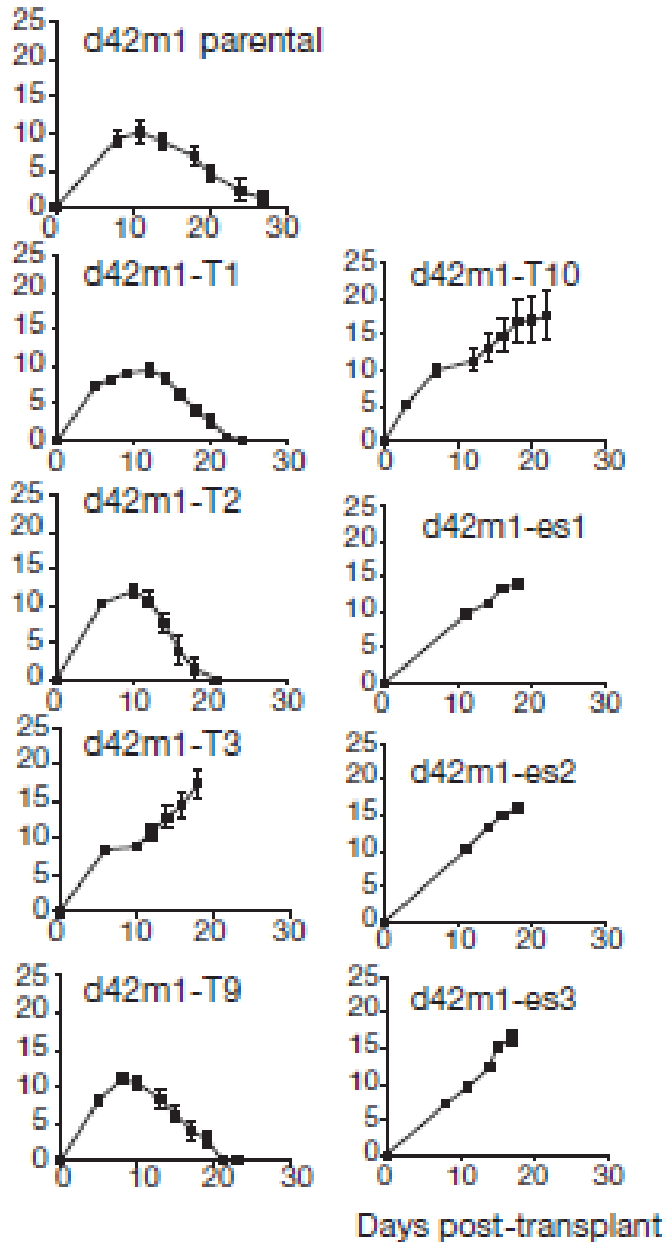
Correspondence

rdschreiber@wustl.edu (R.D.S.), martyomov@wustl.edu (M.N.A.)

In Brief

Comprehensive changes in the tumor microenvironment during successful immune-checkpoint therapy are profiled, implicating a key role for polarization of infiltrating macrophages in the anti-tumor immune milieu.

Mouse model of tumor rejection – panel of sarcomas

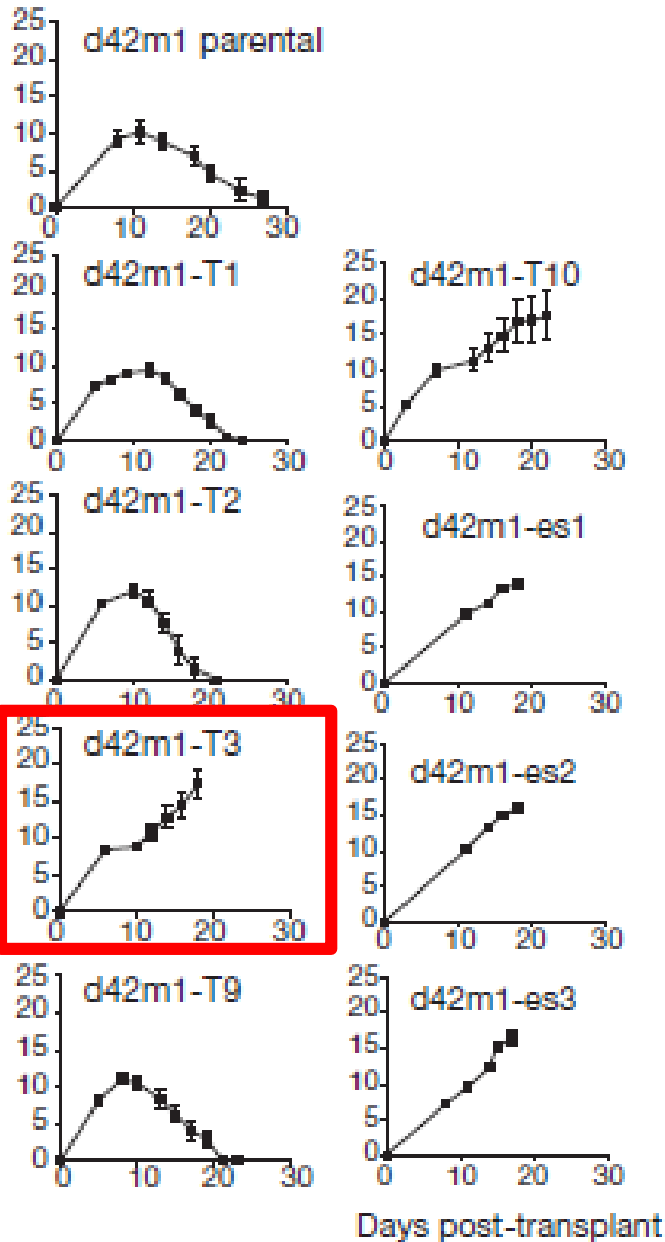


Some mouse sarcomas are naturally rejected while others grow out

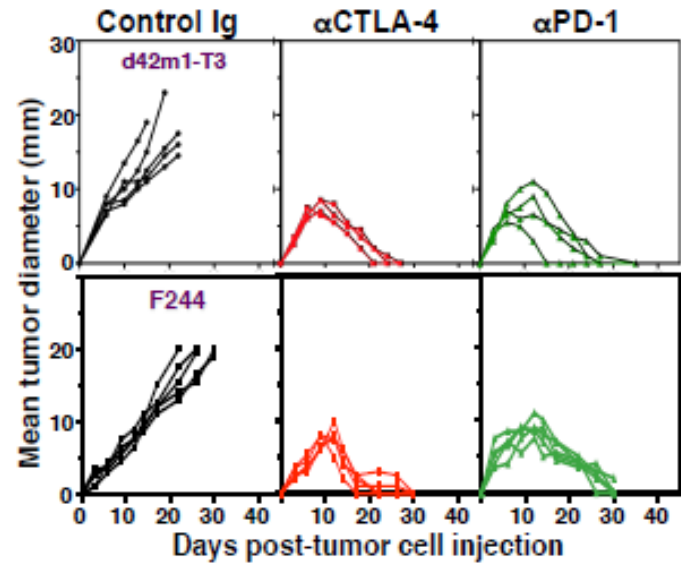
Matsushita et al, Nature 2012
Schreiber lab



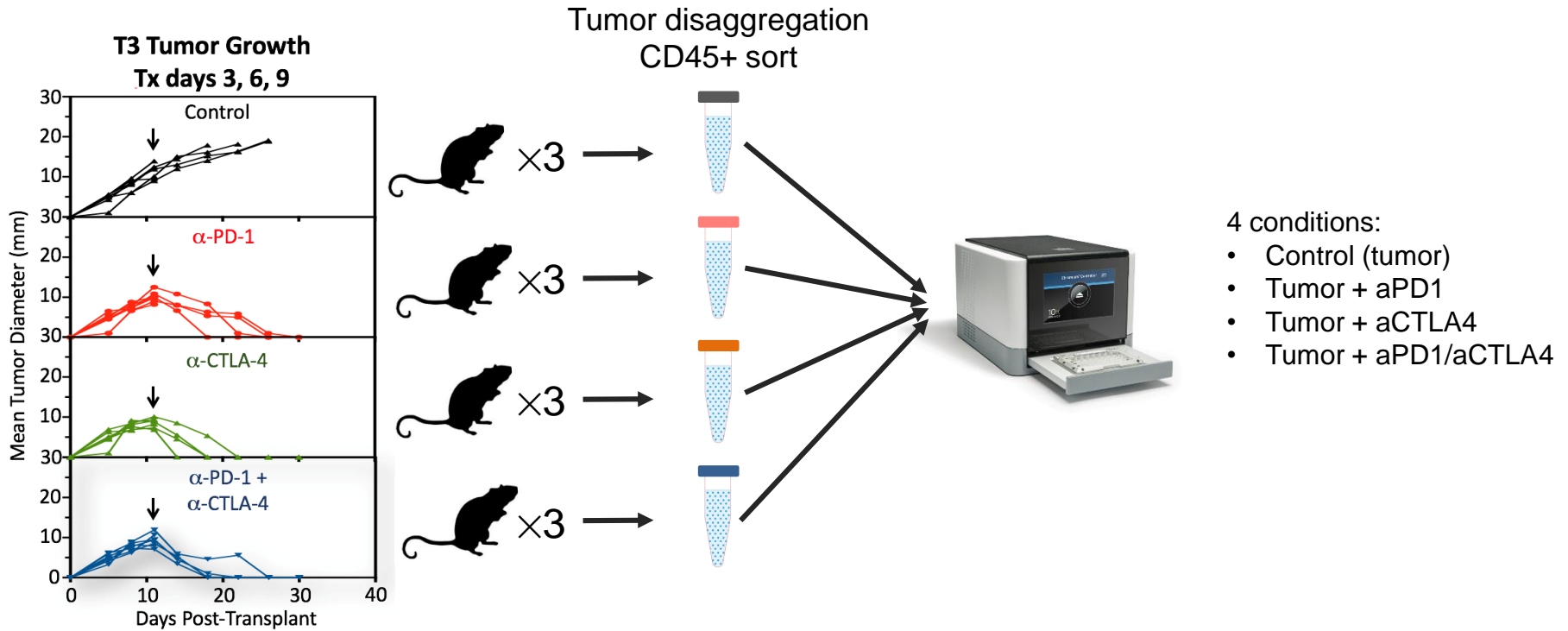
Checkpoint blockade works in progressor tumors



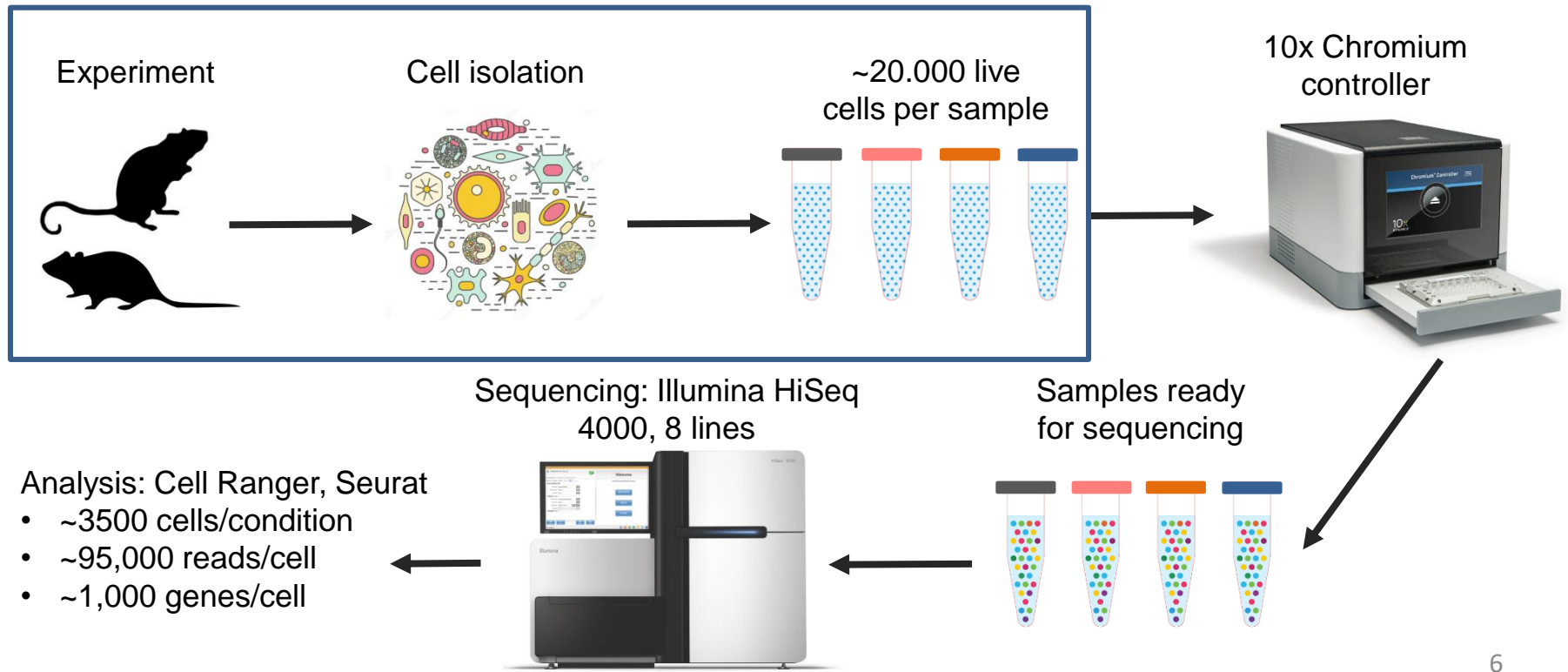
aCTLA4/aPD1 treatments “cure” the mice



scRNA-seq analysis of TIL's

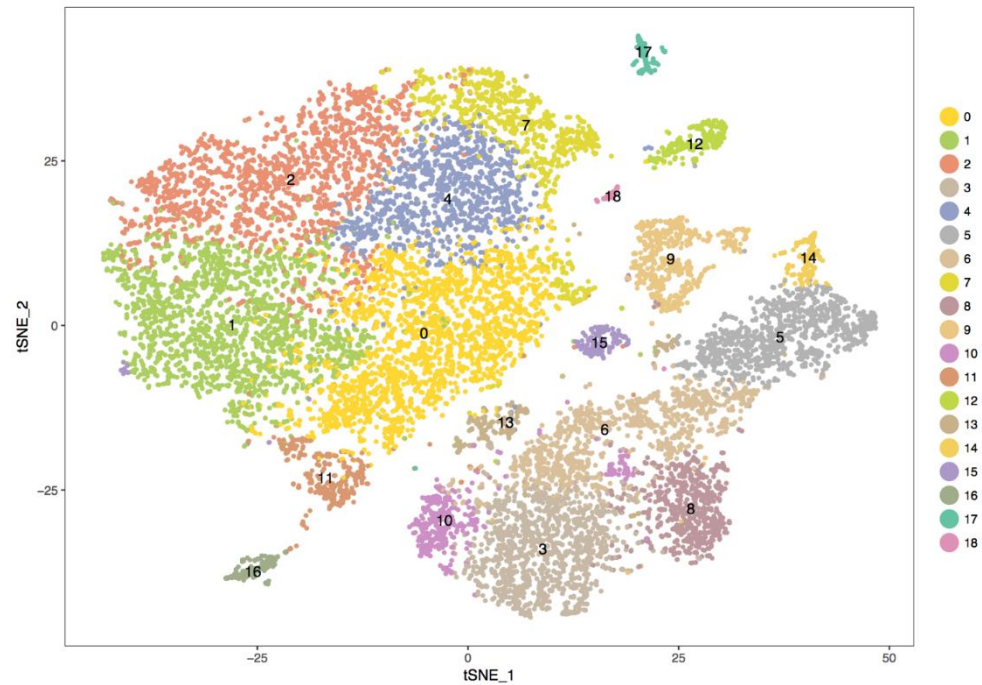
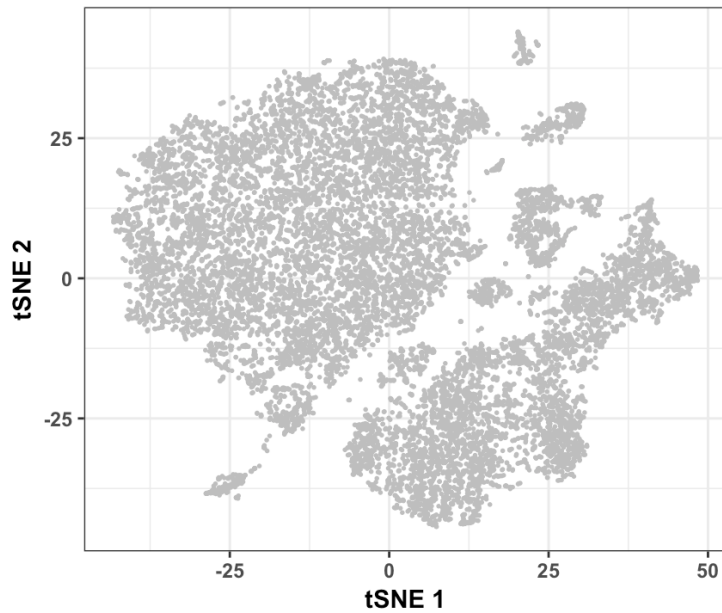


scRNA-seq experimental pipeline



Visualization and clustering: 19 subclusters

14493 cells: 4 conditions together



Datasets

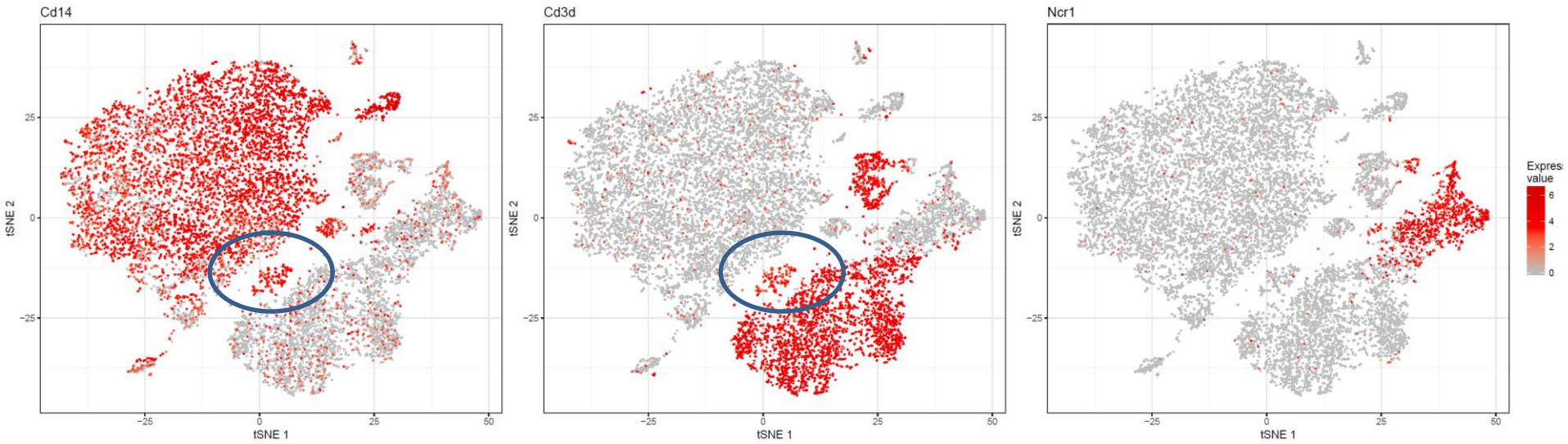
Original layout:

https://artyomovlab.wustl.edu/shiny//single_cell_explorer/?secretToken=schreiber_ss1_engu1aeT

Reprocessed but still good biology:

GSE119352_SRA765288 @ <http://artyomovlab.wustl.edu/sce/>

Note duplicate cluster!
Not a real thing!!!

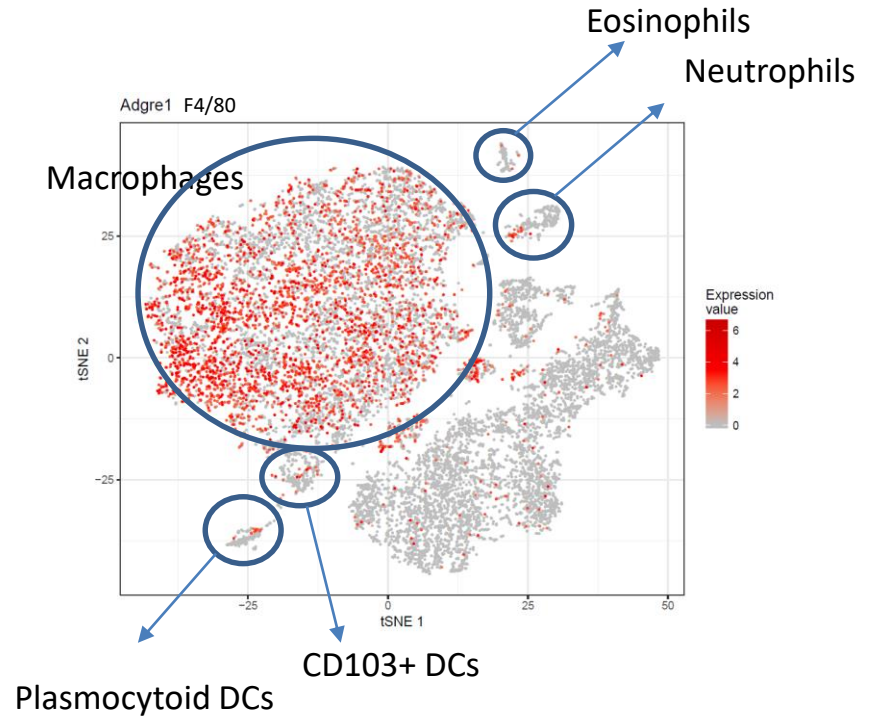
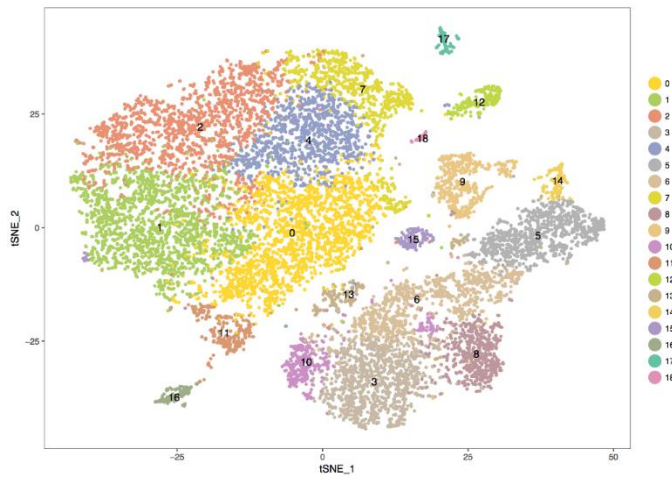


Myeloid

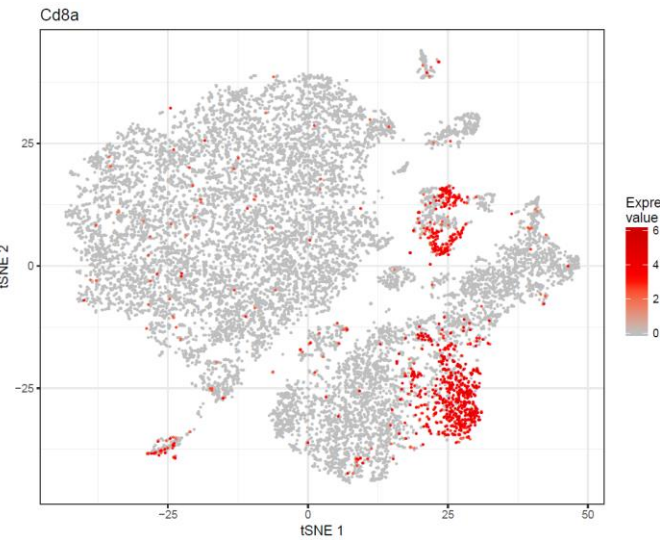
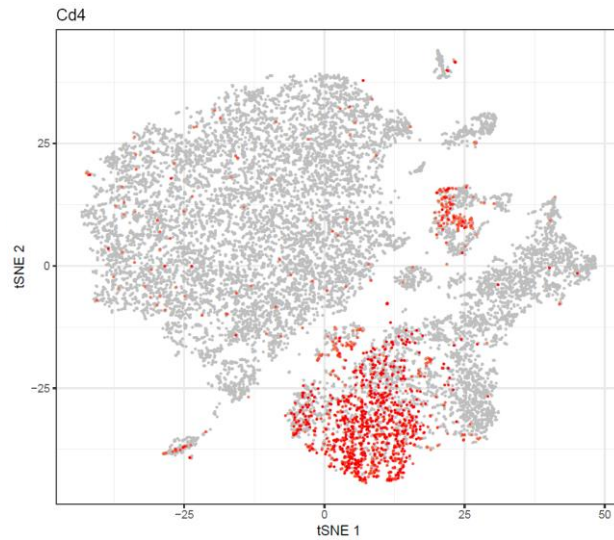
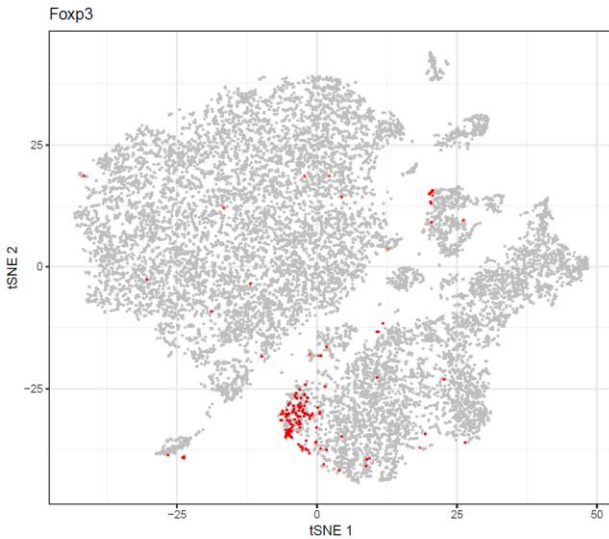
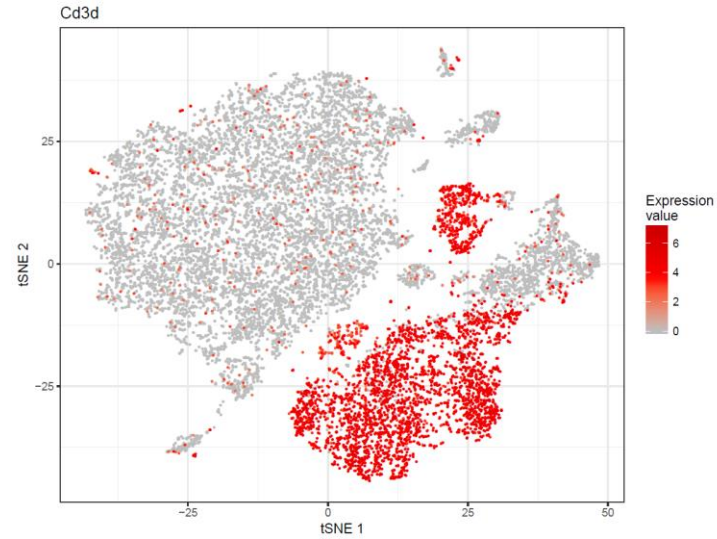
T-cells

NK-cells

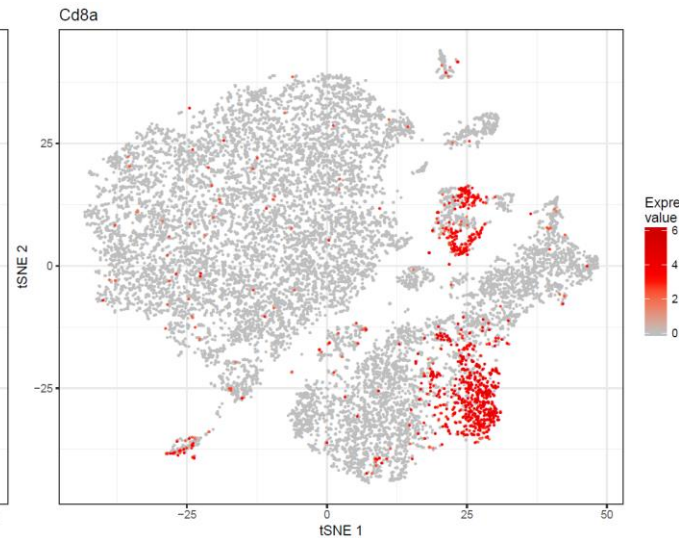
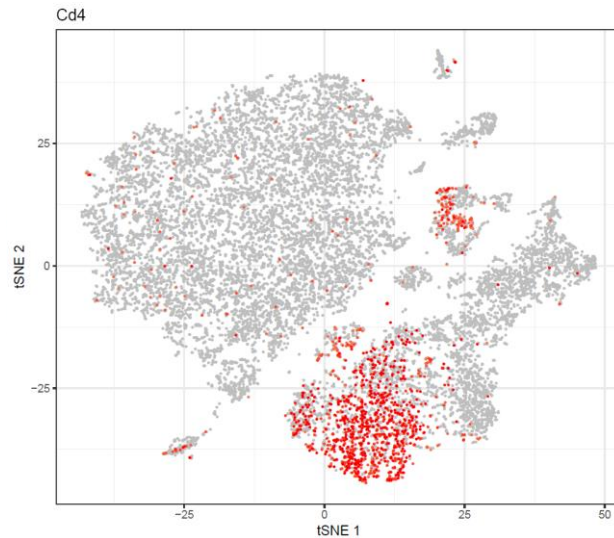
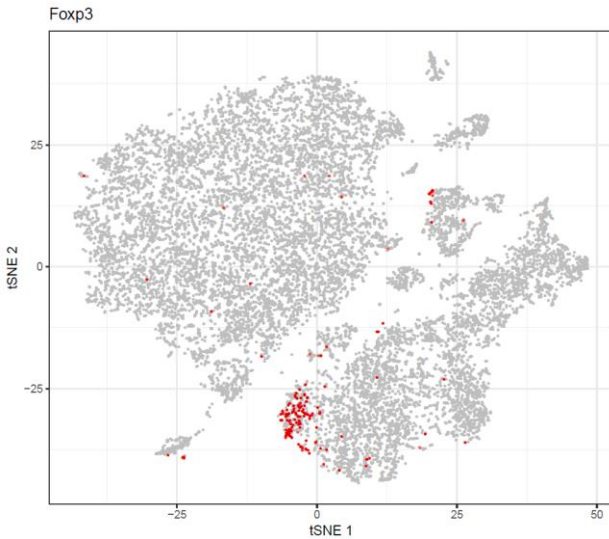
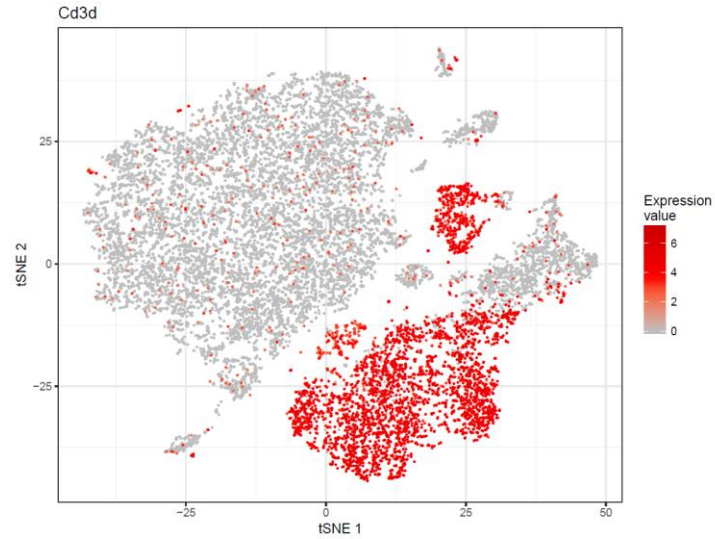
More myeloid subpopulations: Adgre1 (F4/80), Siglech, Cd103, S100a8...



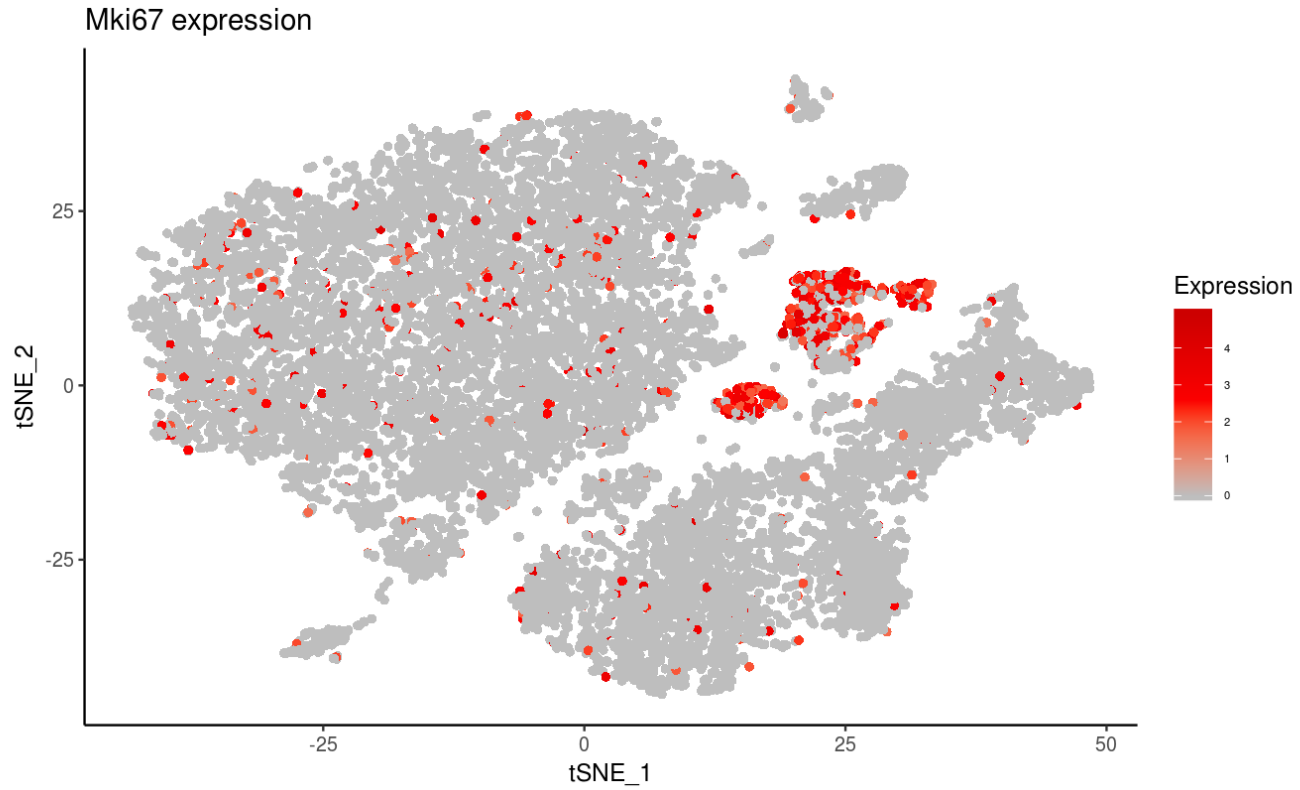
More lymphoid subpopulations: Cd8a, Cd4, Foxp3...



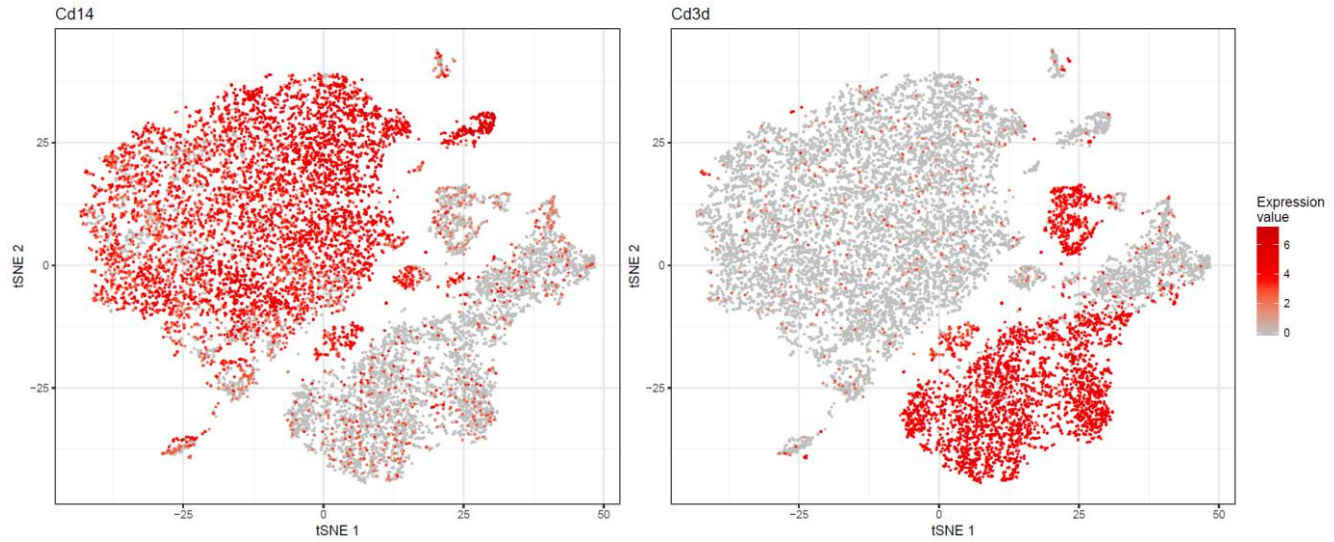
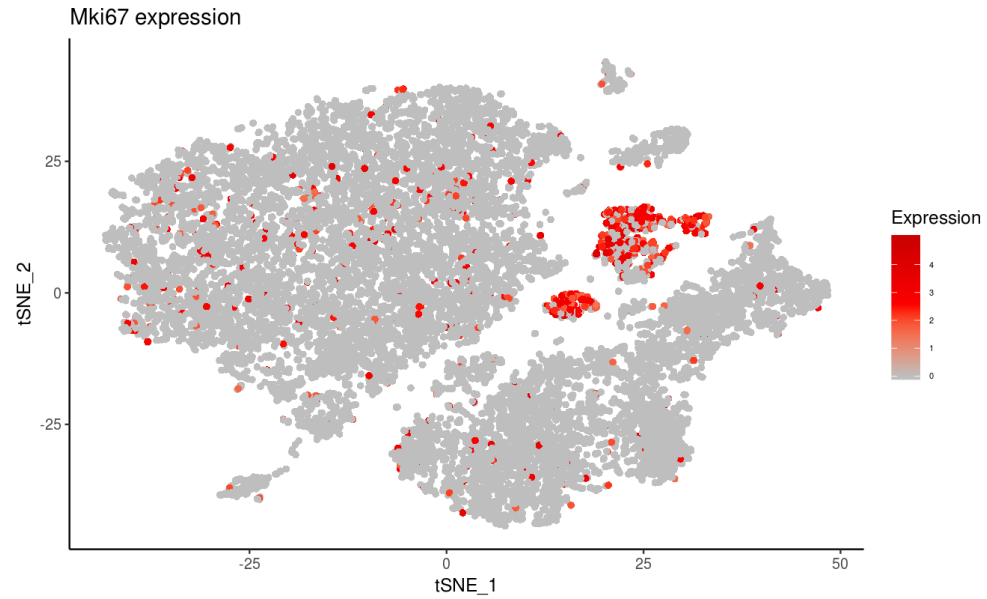
More lymphoid subpopulations: Cd8a, Cd4, Foxp3...



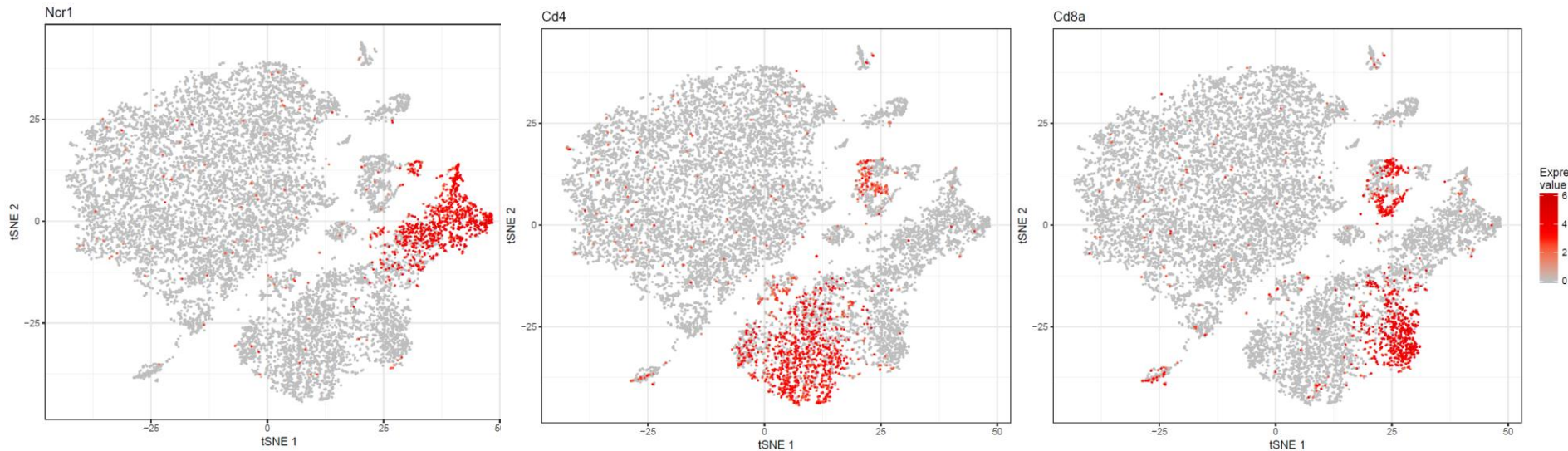
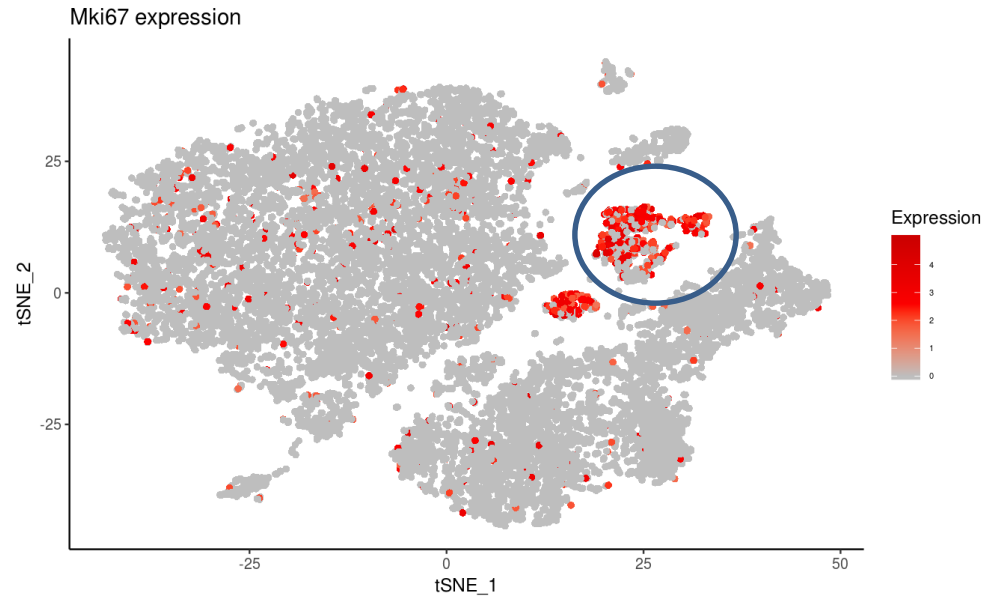
Mki67



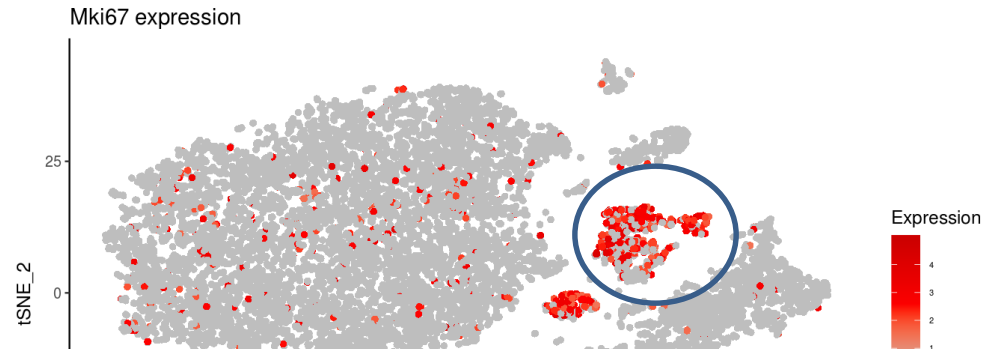
Mki67



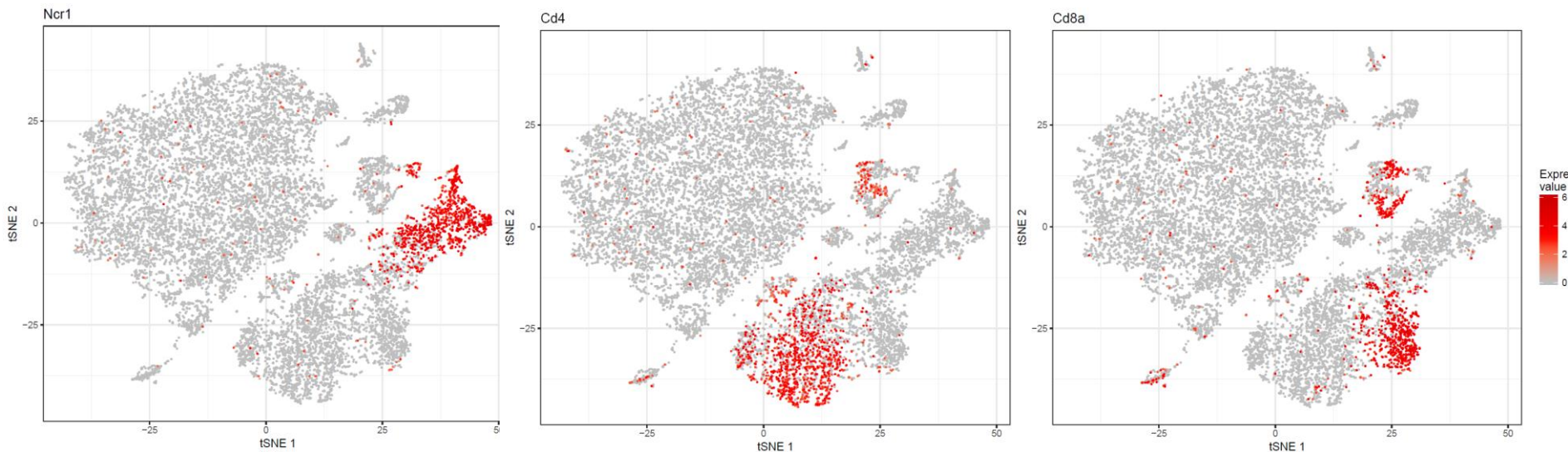
Mki67 lymphoid has Cd4 T + Cd8 T + NK cells



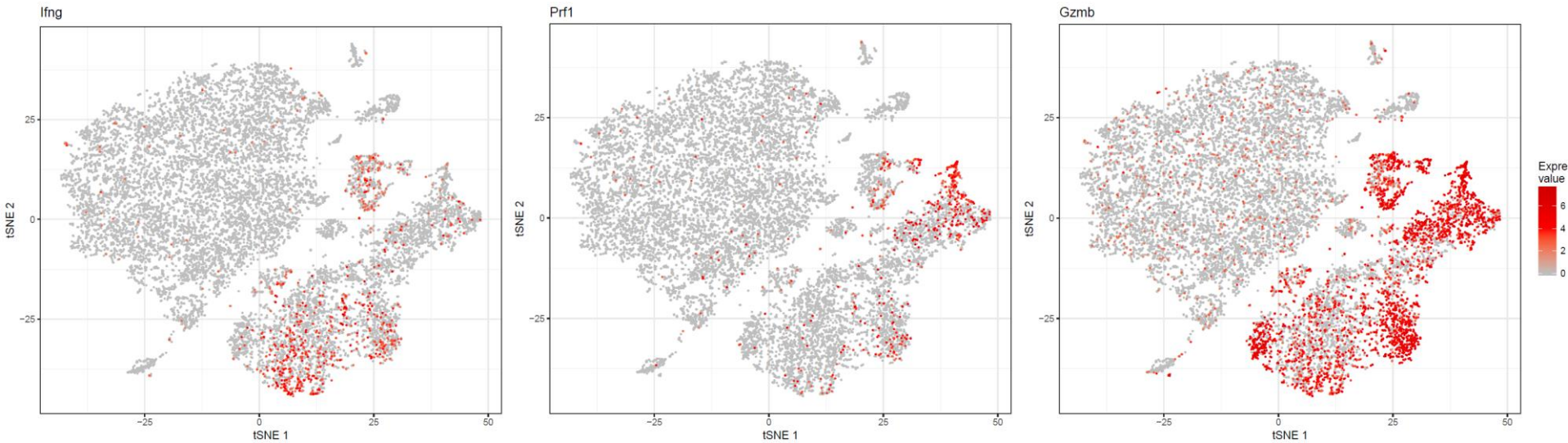
Mki67 lymphoid has Cd4 T + Cd8 T + NK cells



**Take home message:
clustering is mathematical exercise,
it does not prove real biology – it only suggests it!**

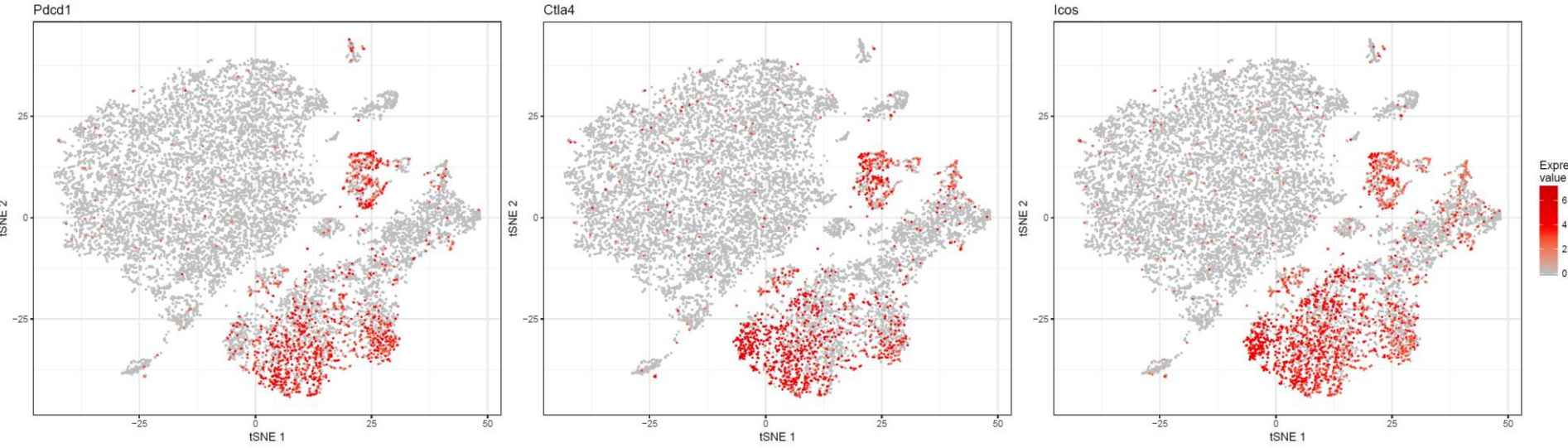


Action markers: Ifng, Prf1, Gzmb

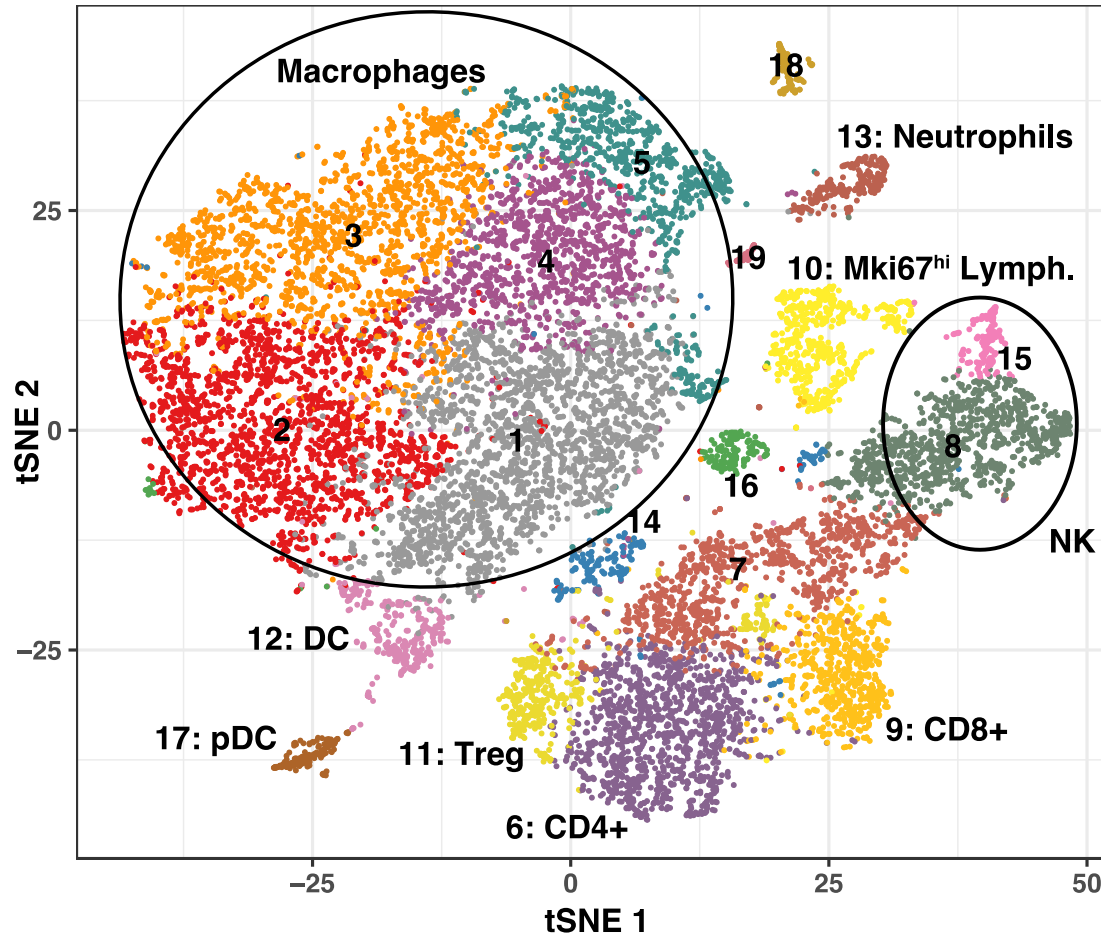


Notice that t-regs are gzmb positive! (first shown by tim ley)

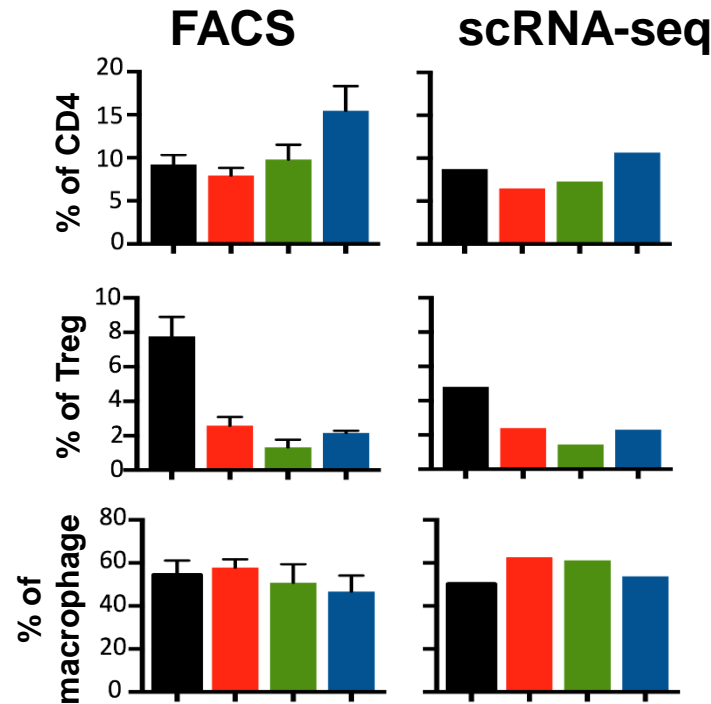
Checkpoint markers: Pdc1, Ctla4, Icos...



Subpopulation structure in our data

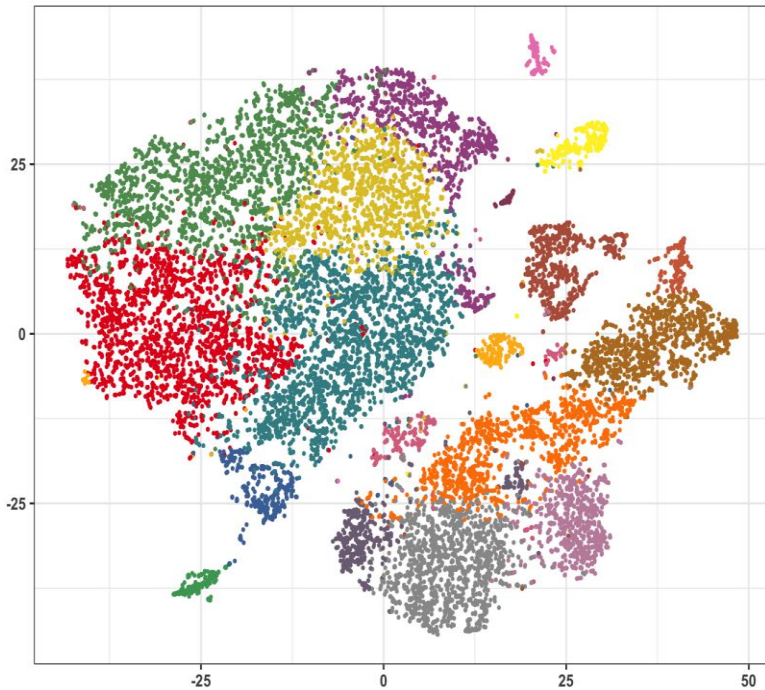


Is scRNA-seq accurately tracking populations?

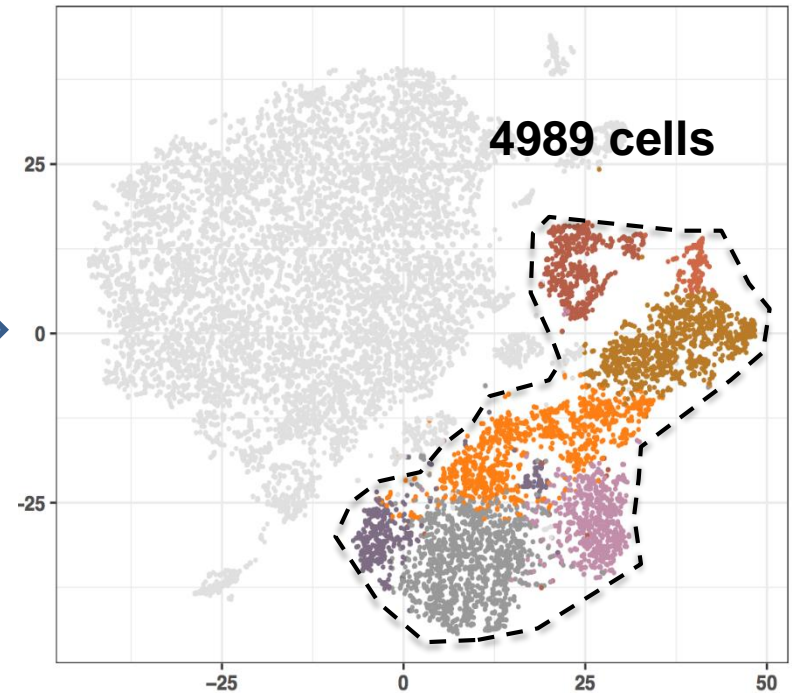


Zoom in on Lymphoid Compartment (scRNAseq)

14493 cells

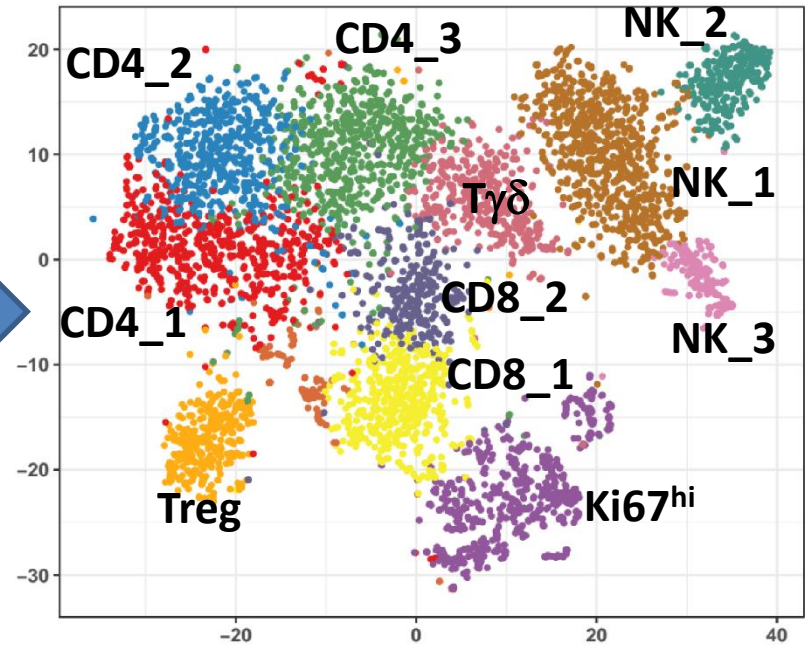
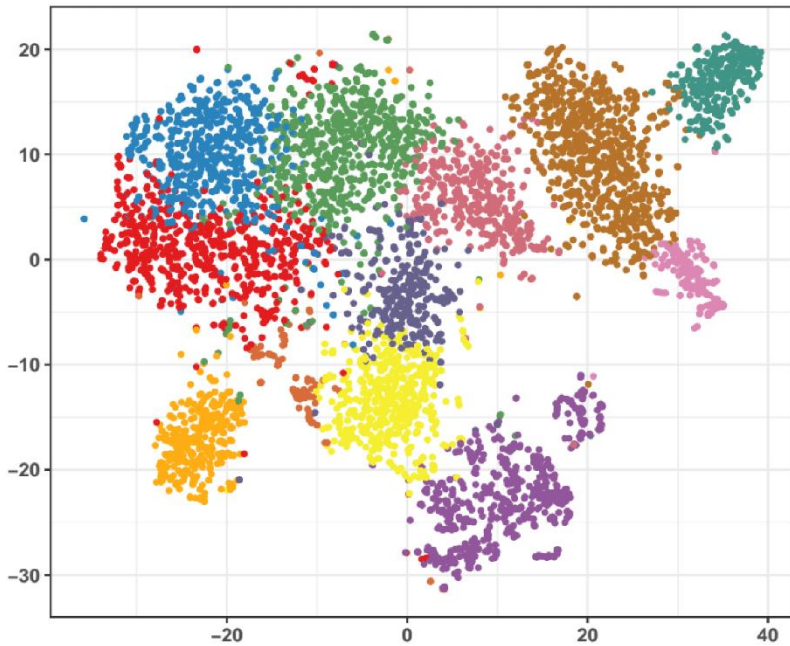


4989 cells



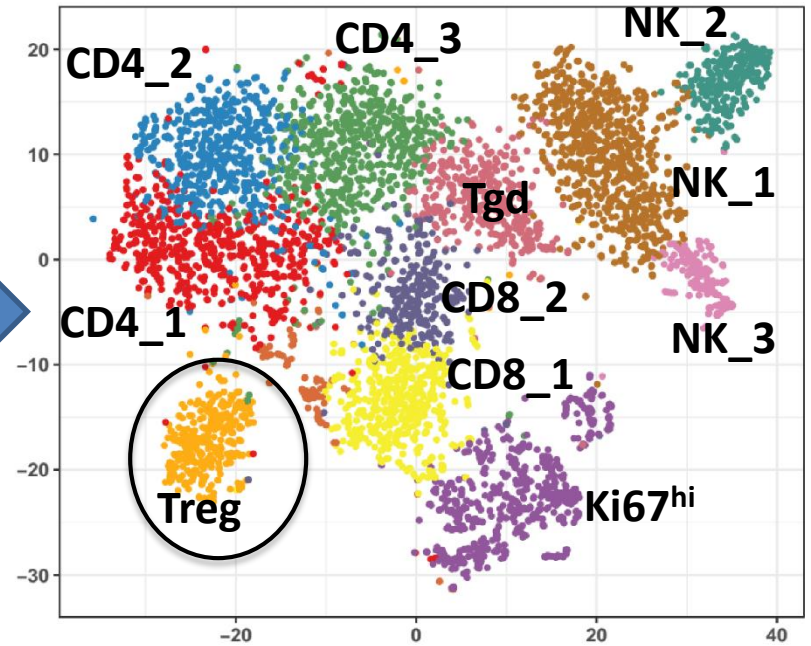
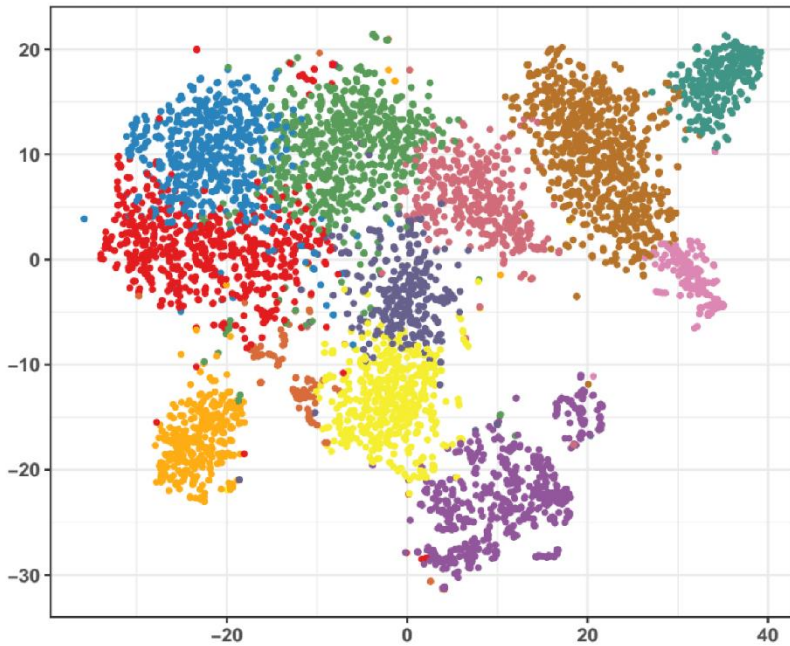
Lymphoid Compartment Annotation (scRNAseq)

Lymphoid: 4989 cells

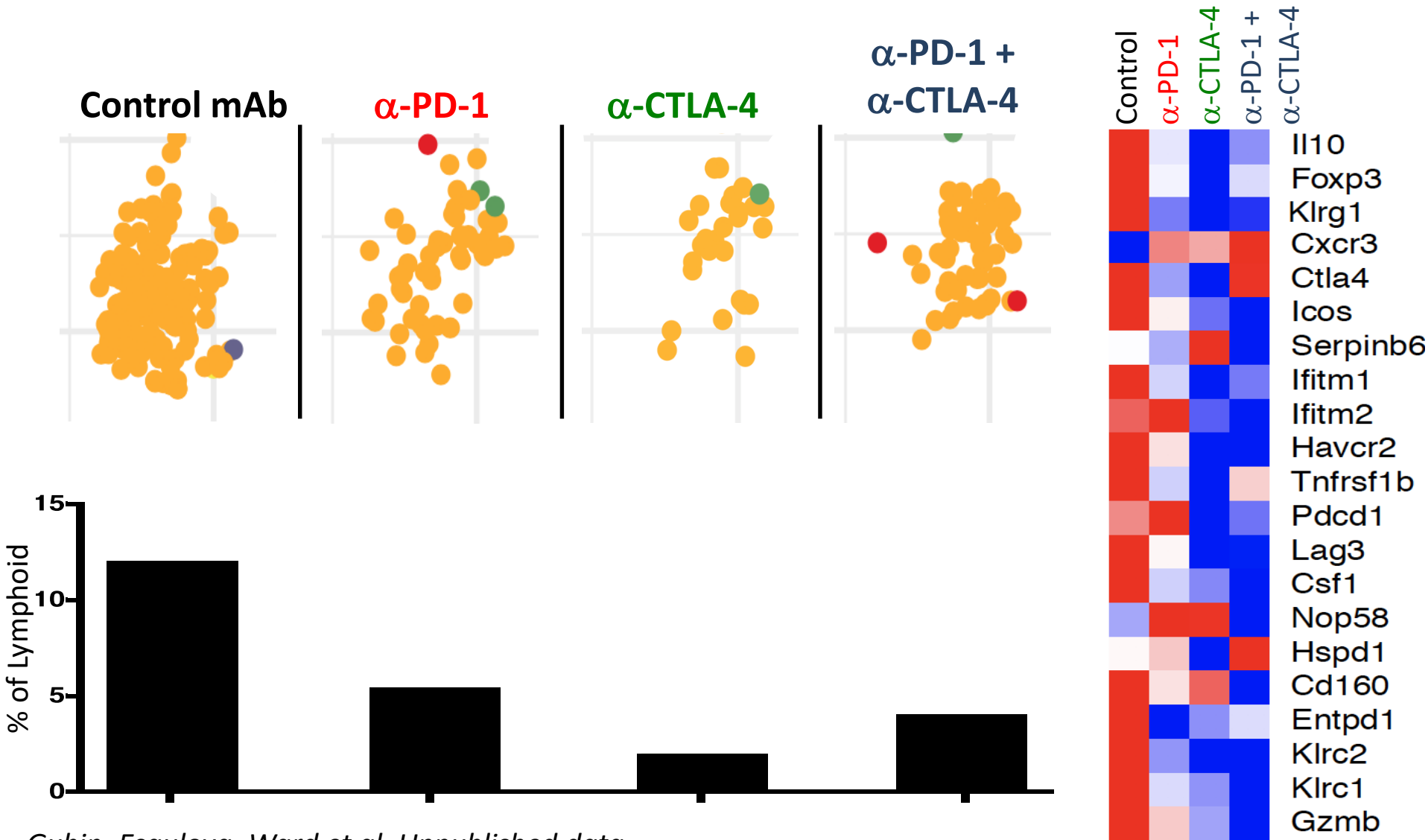


Lymphoid Compartment Annotation (scRNAseq)

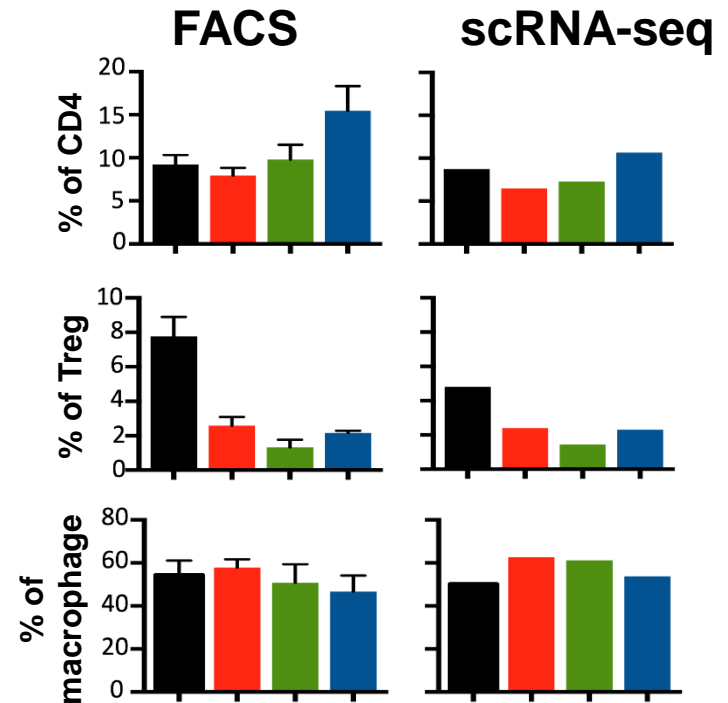
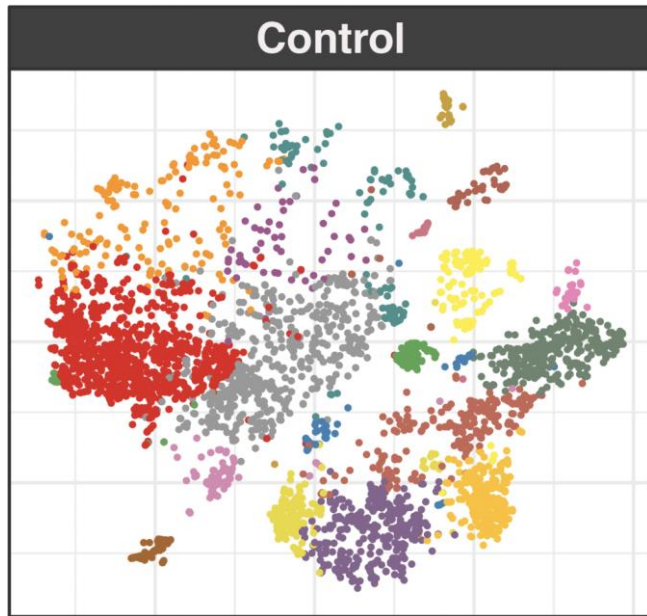
Lymphoid: 4989 cells



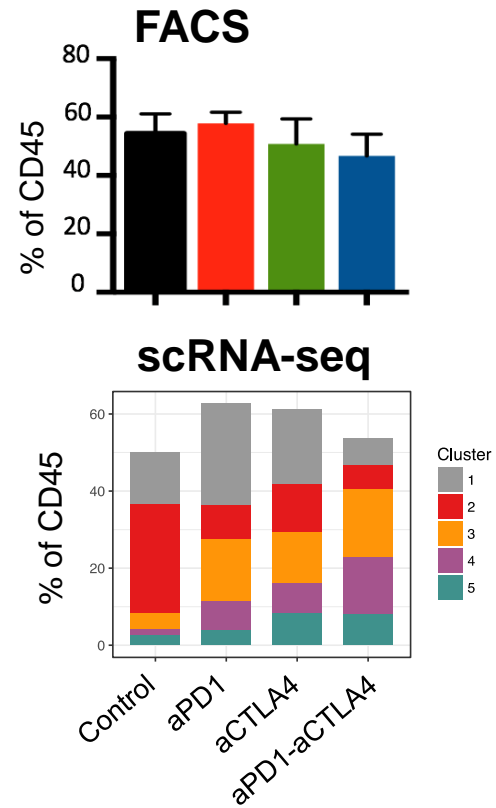
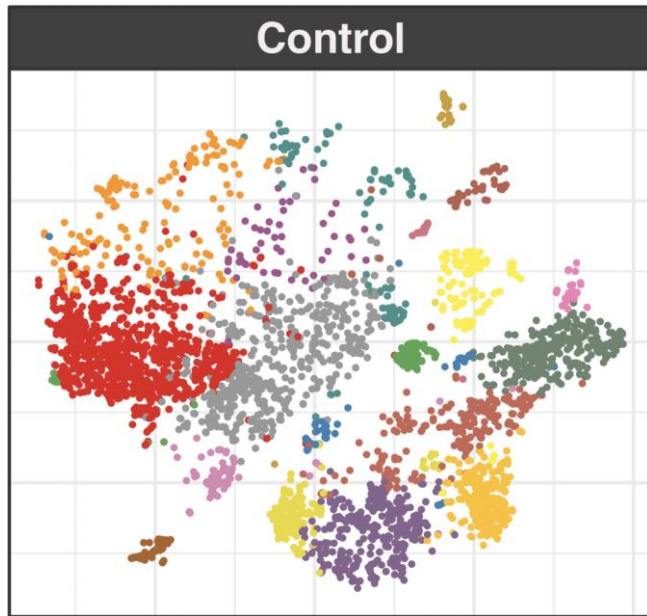
Remodeling of Intratumoral Tregs Upon Immune Checkpoint Blockade Therapy (scRNAseq)



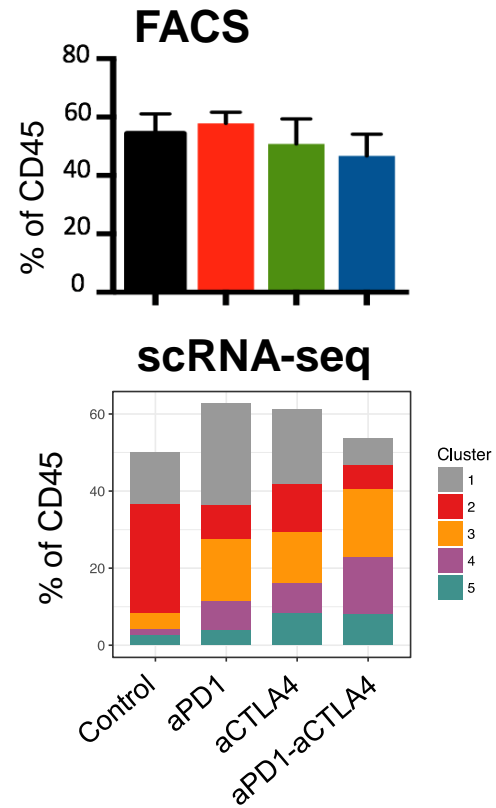
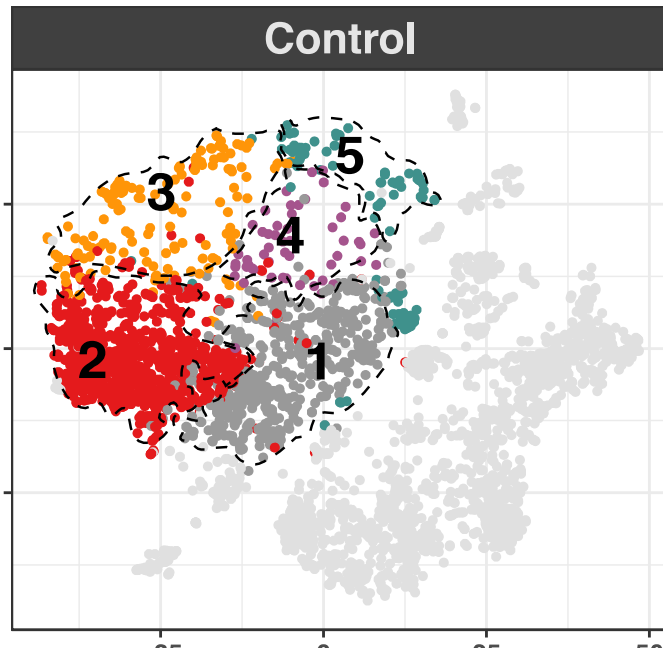
No only lymphoid cells, but also macrophages have treatment associated changes



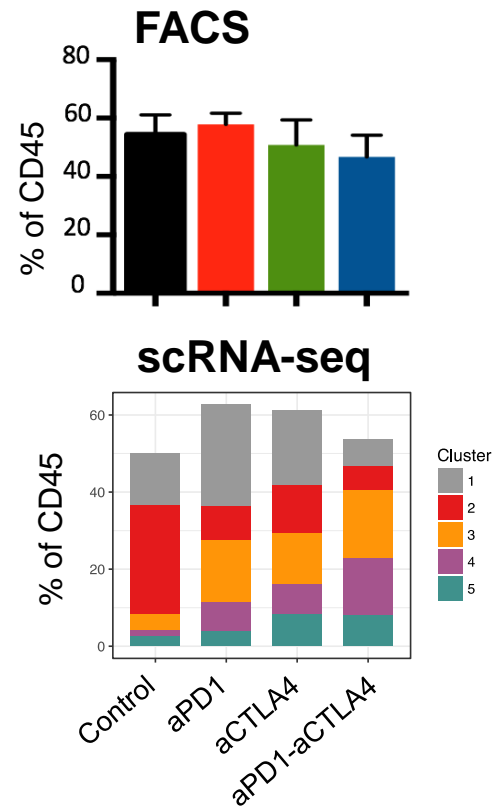
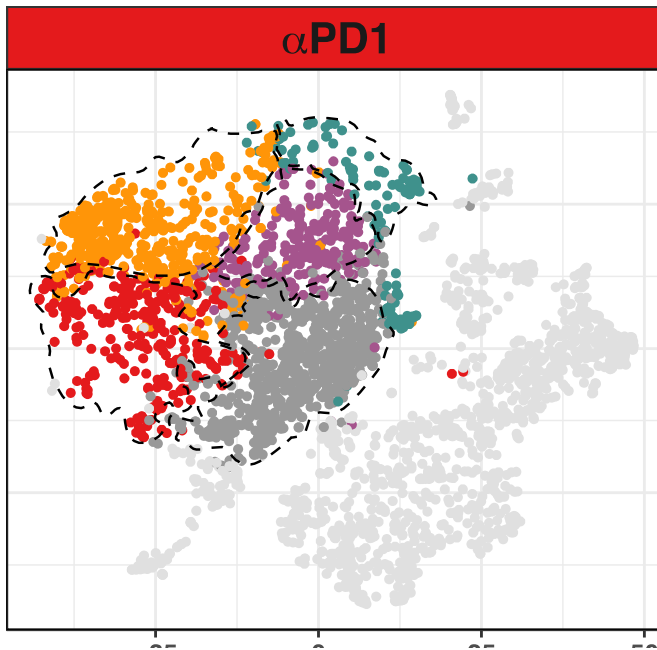
Macrophage subpopulations undergo changes, but overall fraction does not change



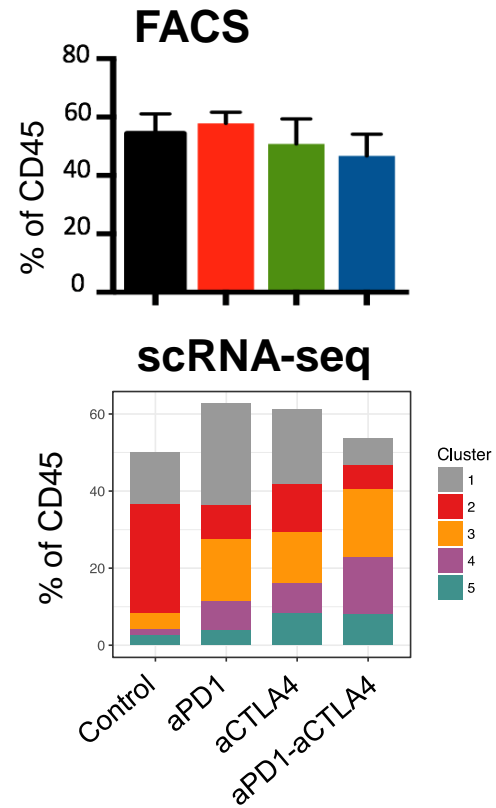
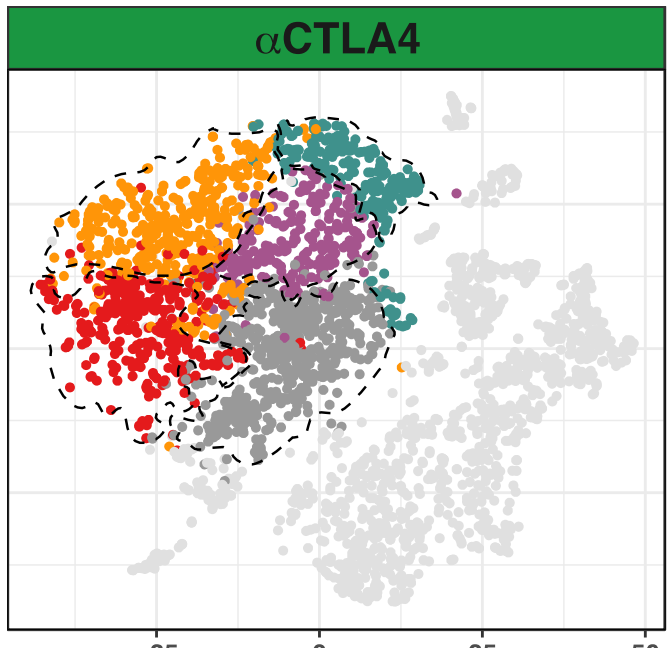
Macrophage subpopulations undergo changes, but overall fraction does not change



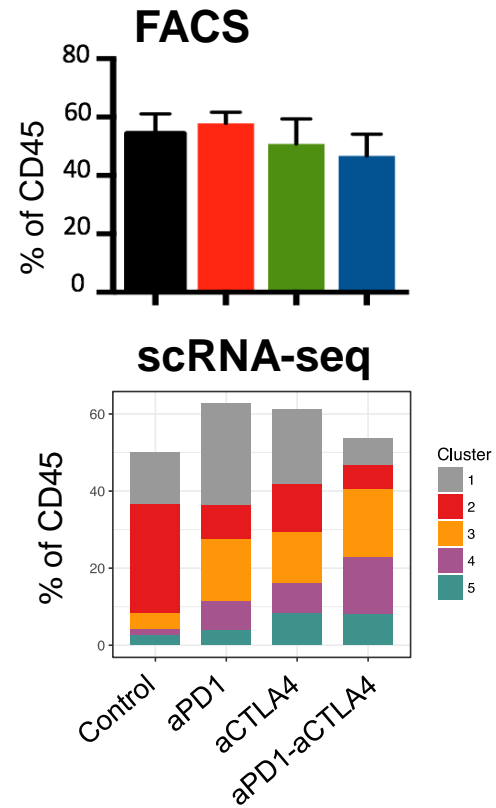
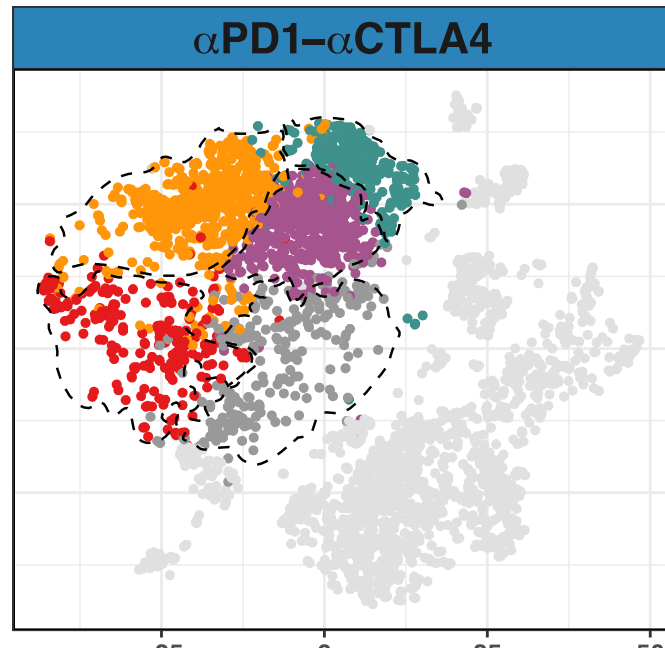
Macrophage subpopulations undergo changes, but overall fraction does not change



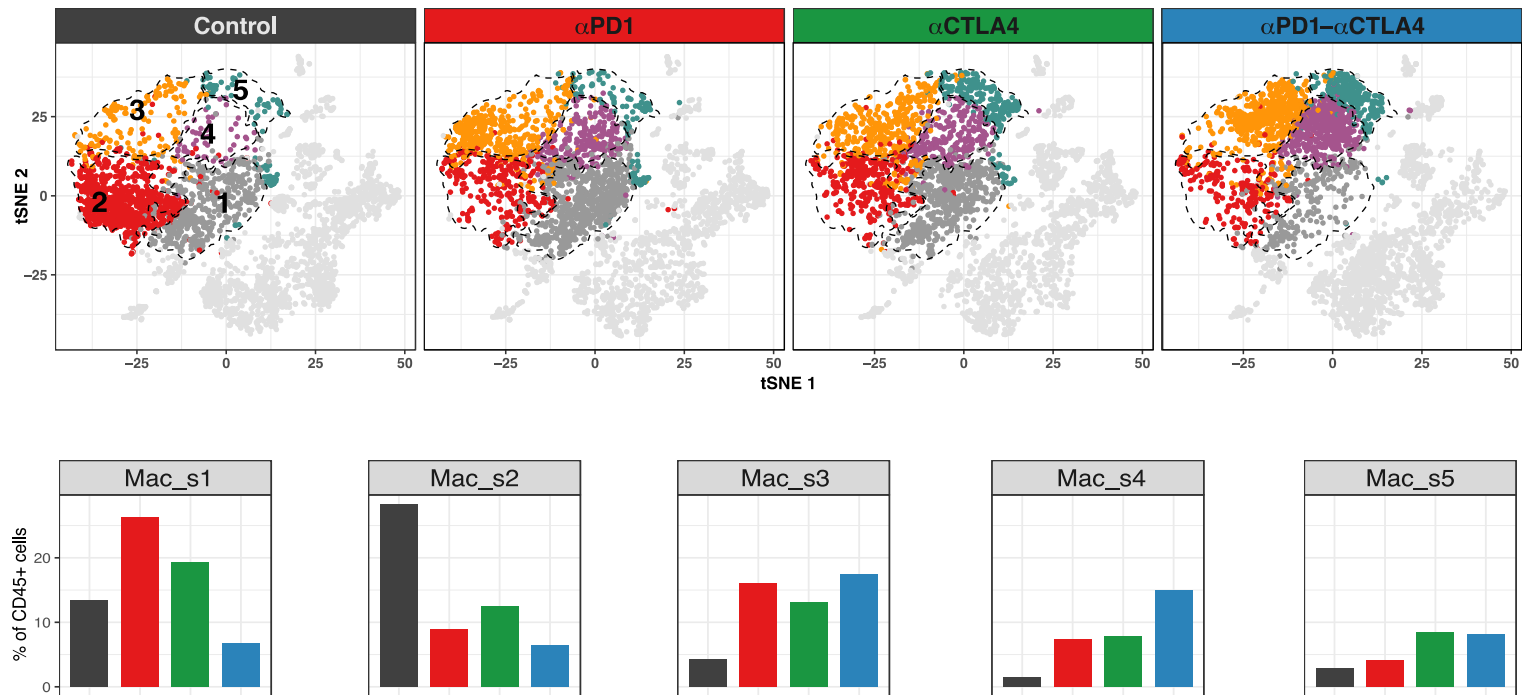
Macrophage subpopulations undergo changes, but overall fraction does not change



Macrophage subpopulations undergo changes, but overall fraction does not change

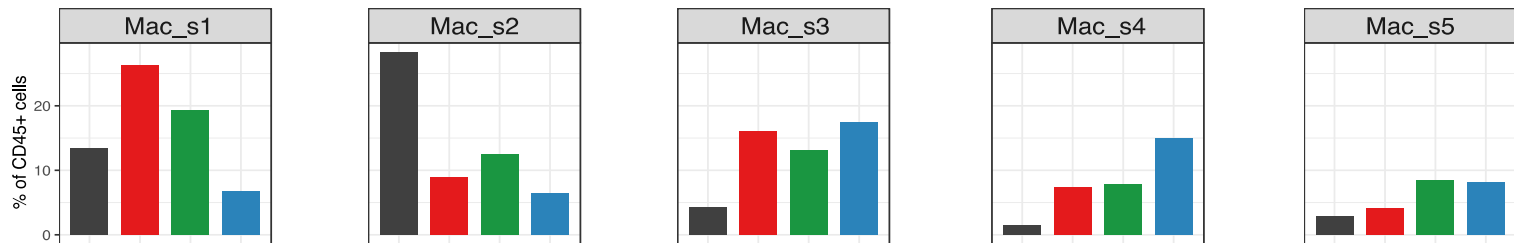


All macrophage clusters change with treatment

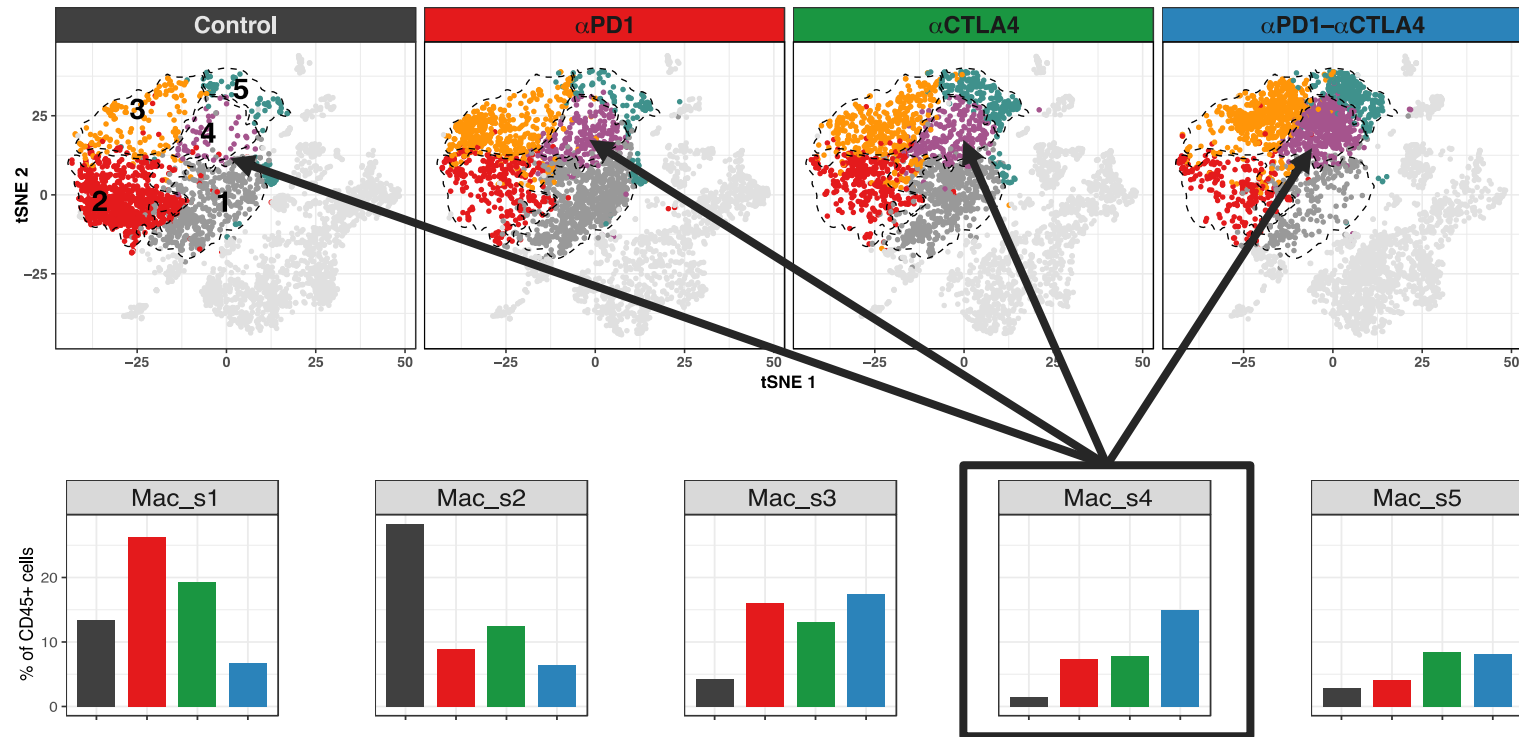


All macrophage clusters change with treatment

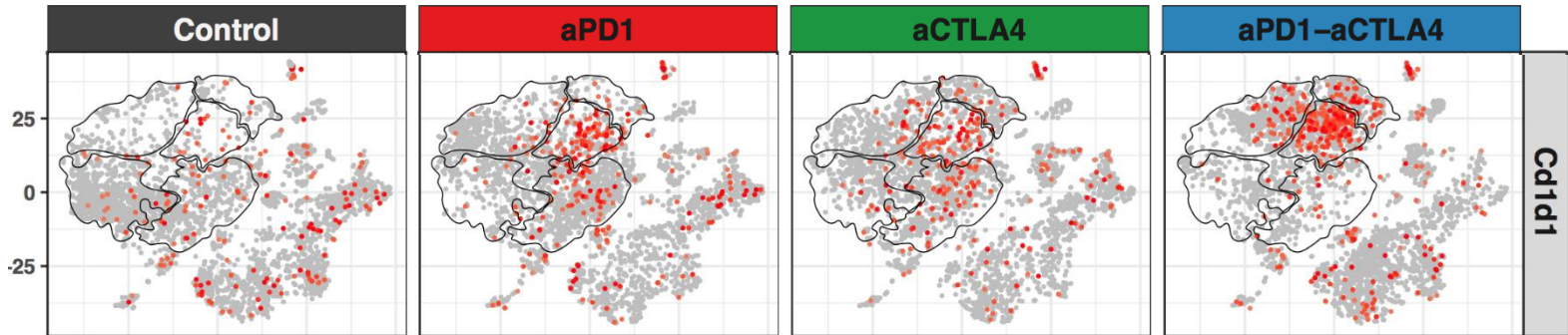
What are these clusters?
Are they real?



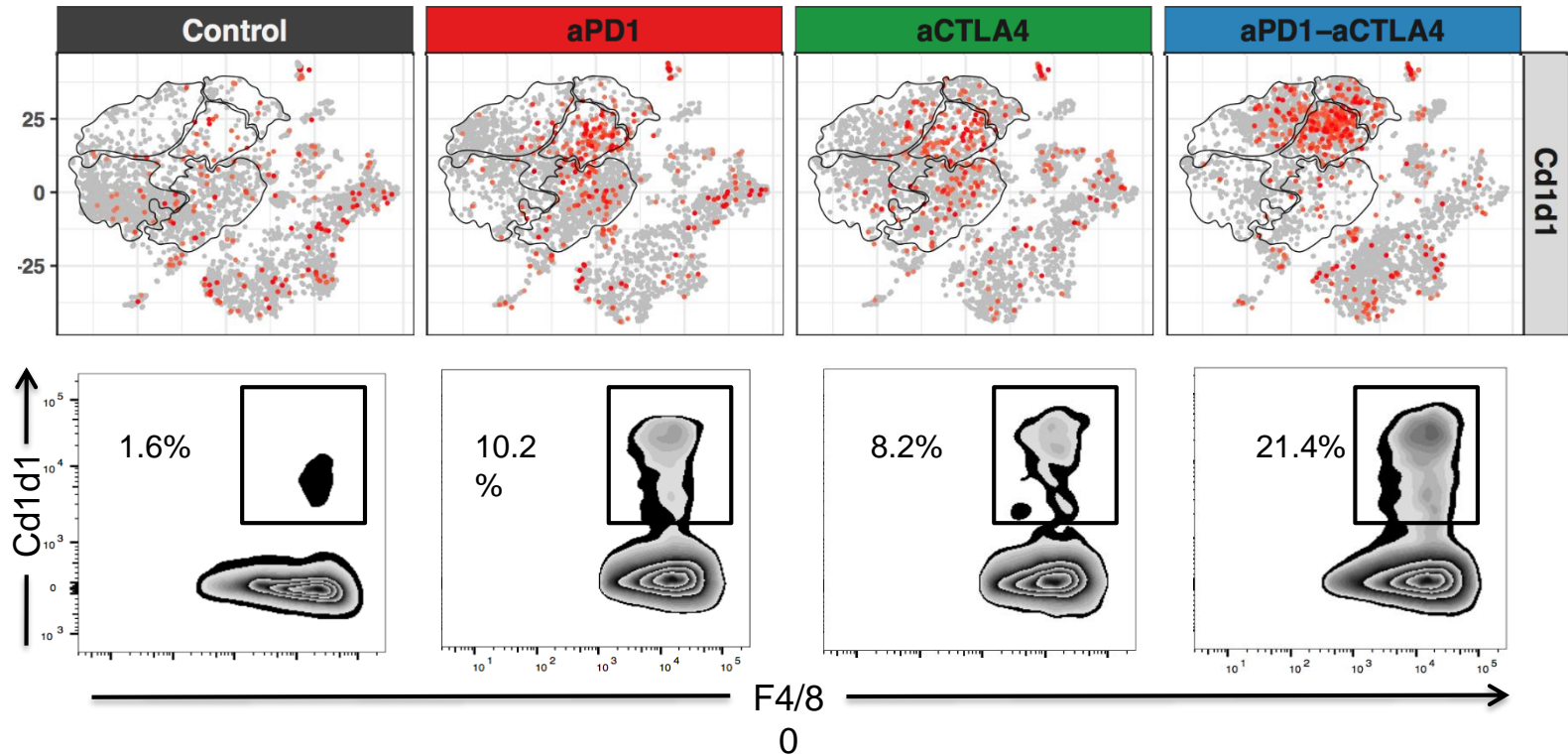
Cluster 4: pro-inflammatory macrophages



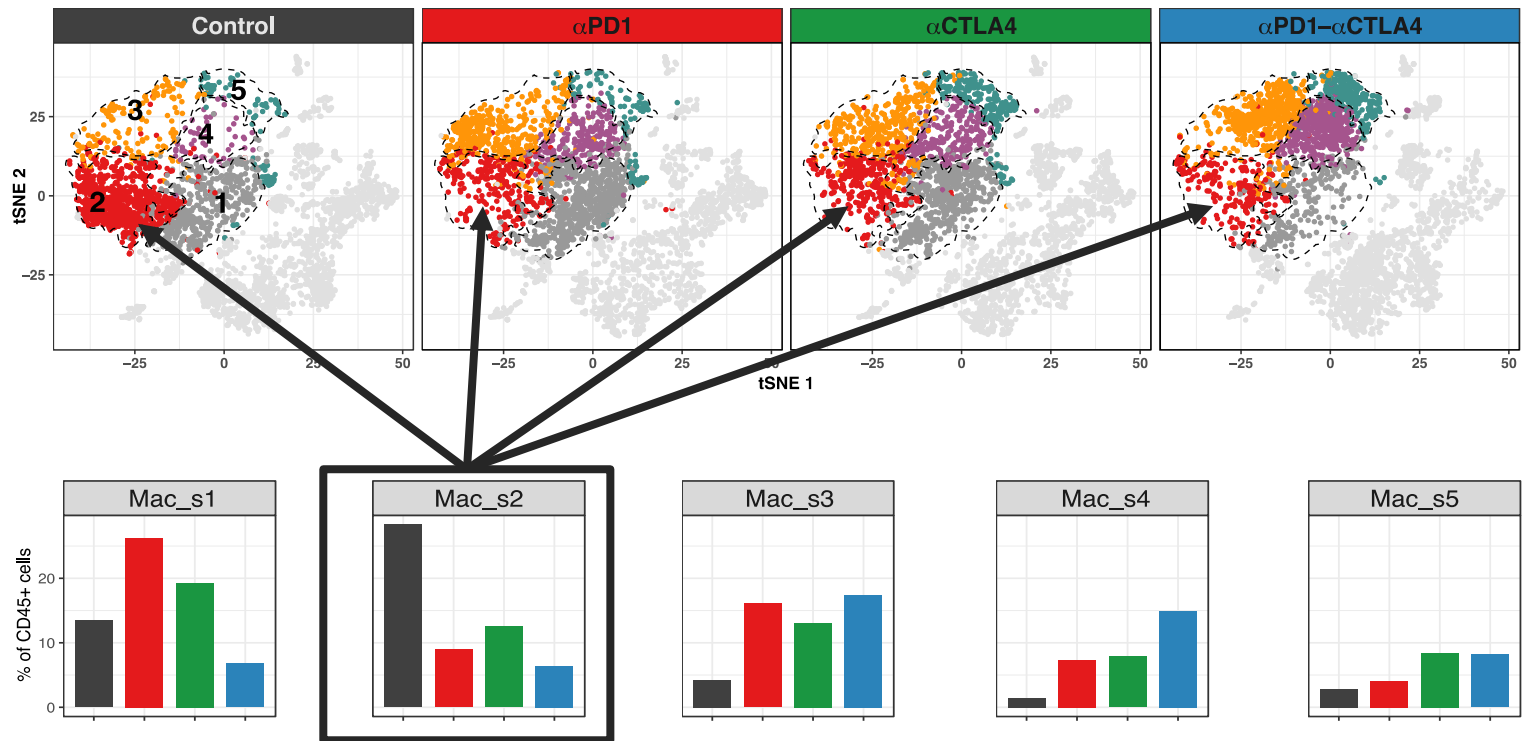
Cluster 4 is defined by exclusive *CD1d1* expression



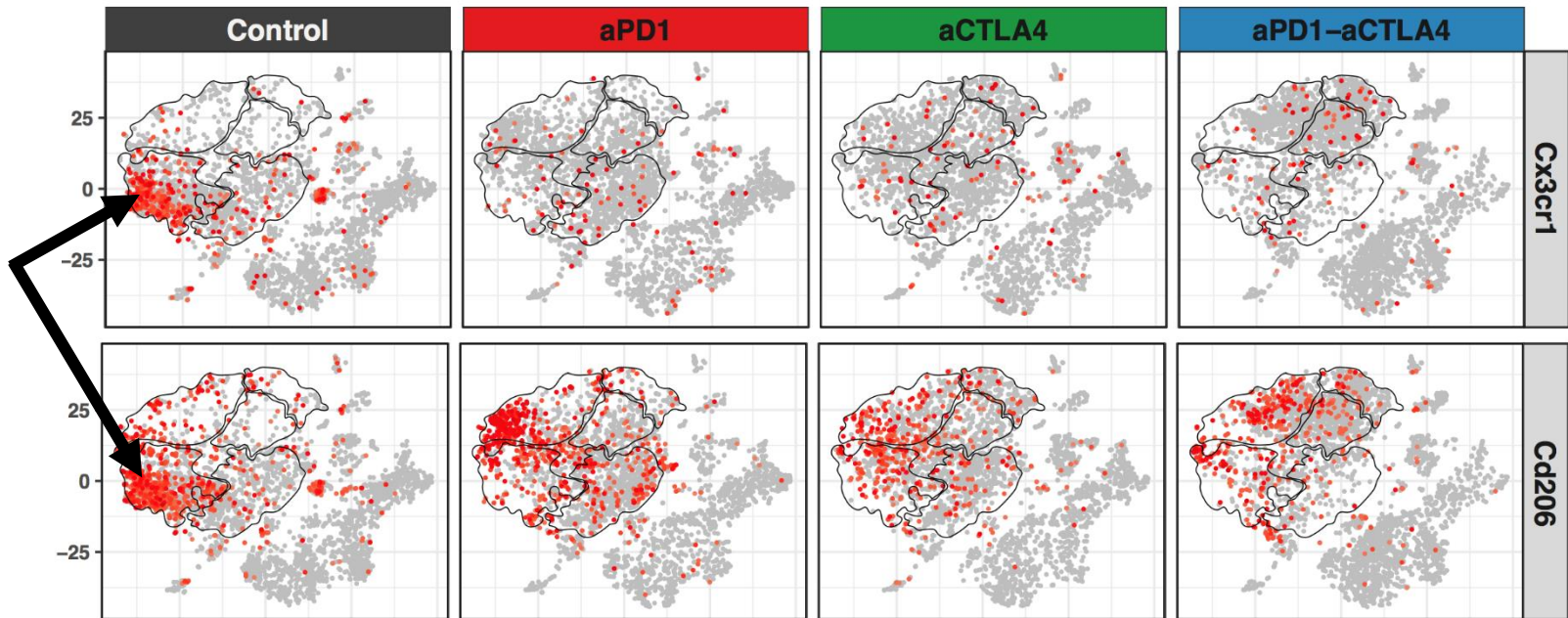
Cluster 4 is defined by exclusive *CD1d1* expression



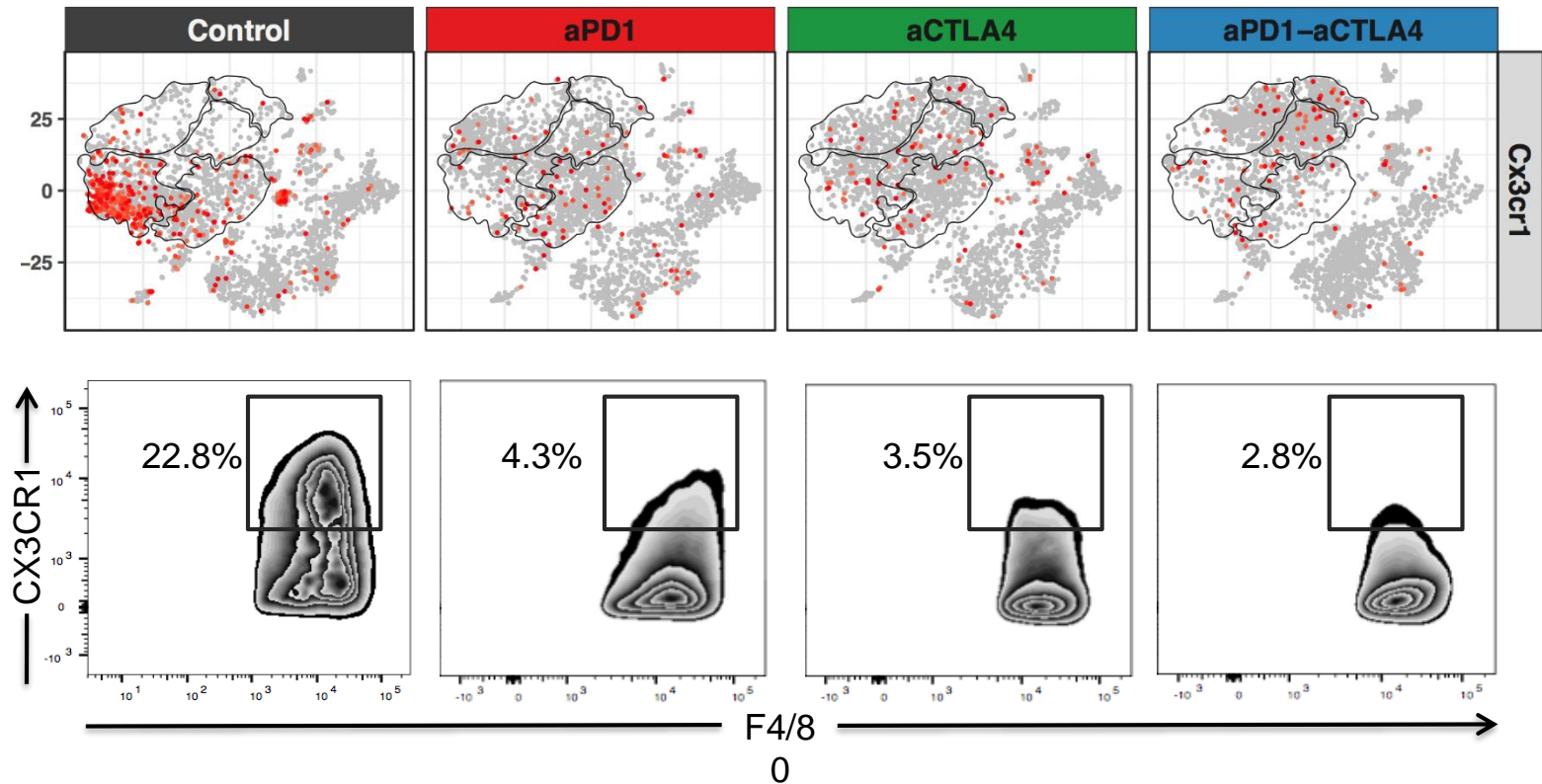
Cluster 2: anti-inflammatory macrophages



Cluster 2: expression of *Mrc1* (*CD206*) and exclusive expression of *Cx3cr1*

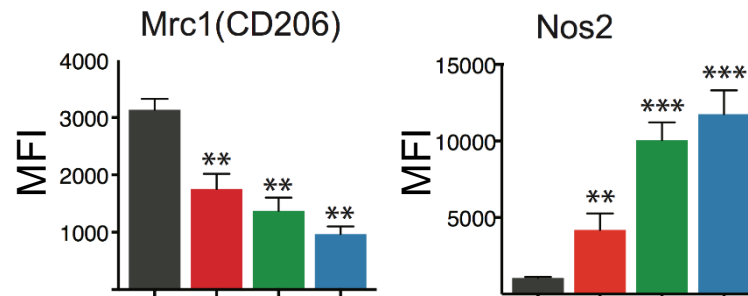
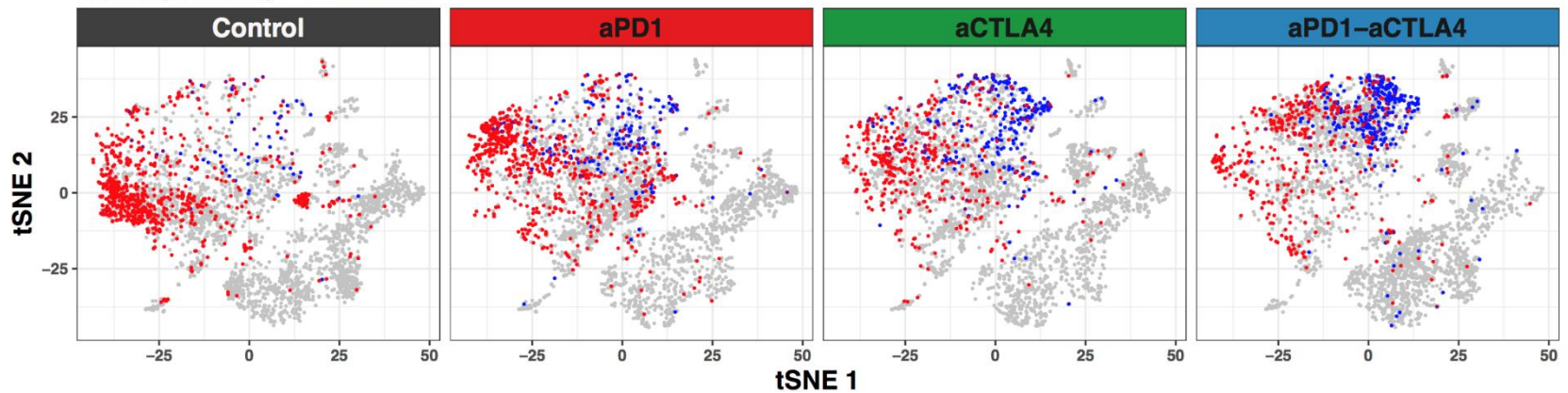


Cluster 2: expression of *Mrc1* (CD206) and exclusive expression of *CX3CR1*

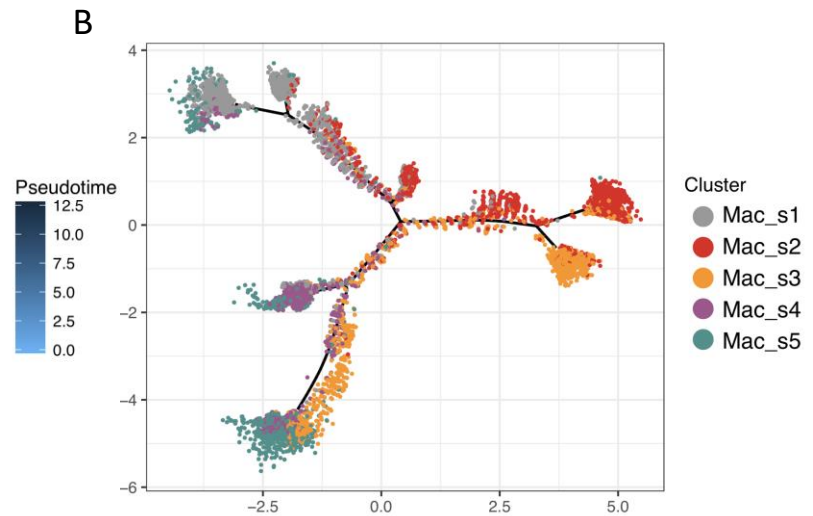
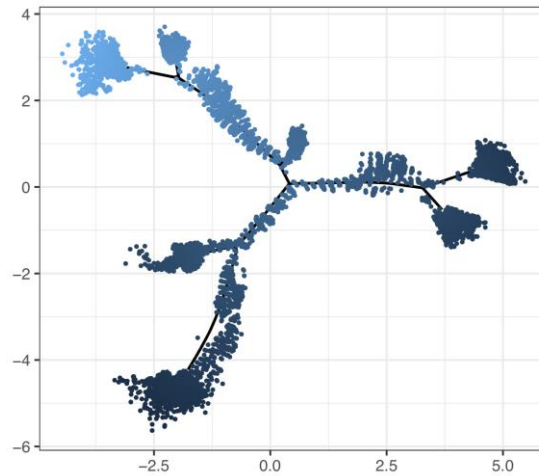
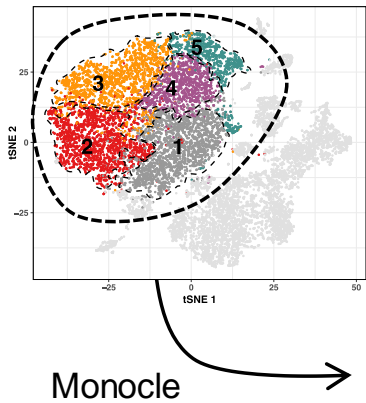


Transition from anti-inflammatory to pro-inflammatory macrophages

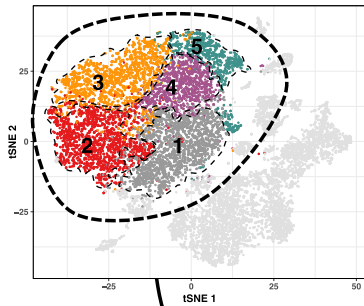
Mrc1 (CD206) and **Nos2**



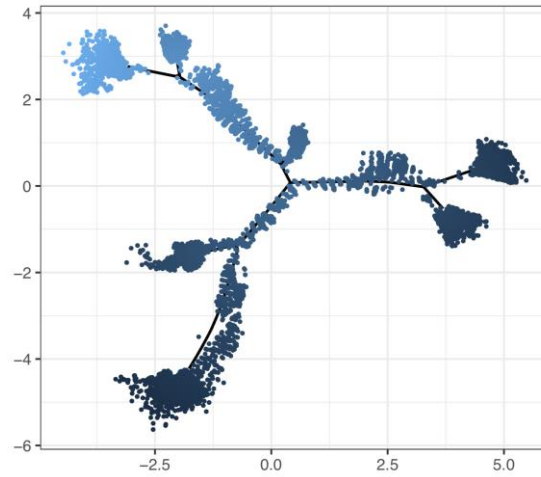
Pseudotime suggest that cluster 1 is the “point of origin”



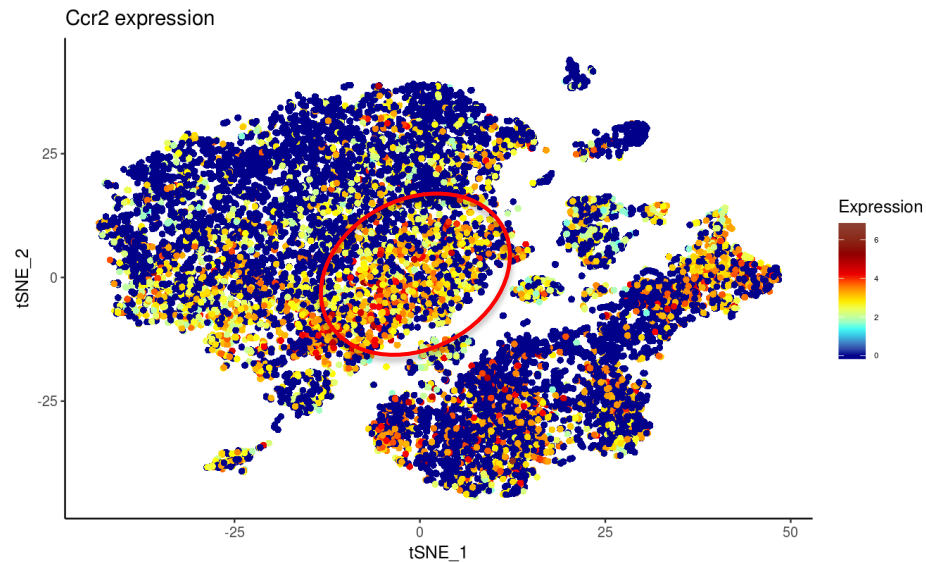
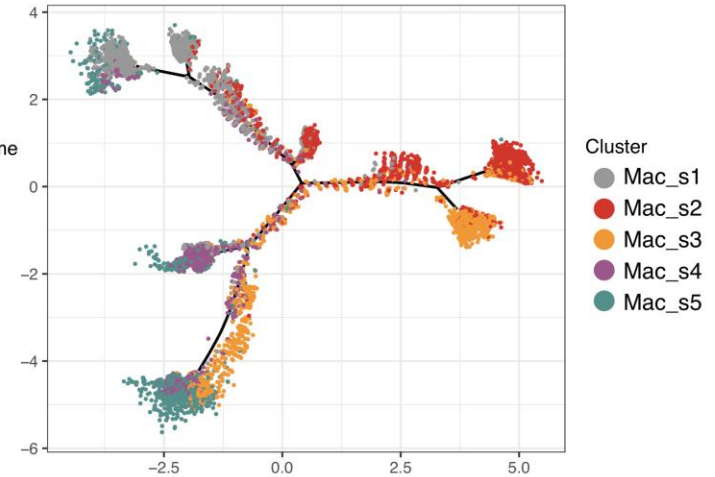
Starts from Ccr2+ population - monocytes



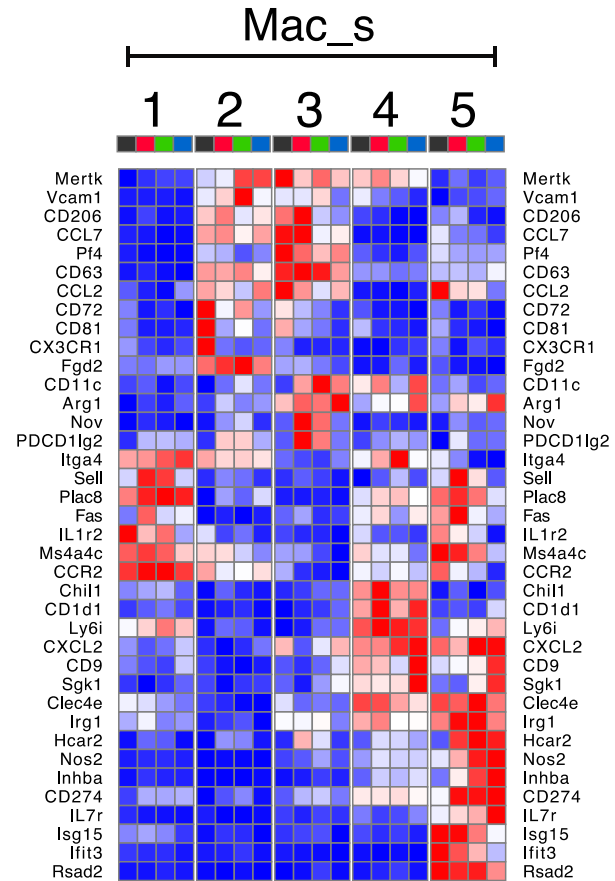
Monocle



B

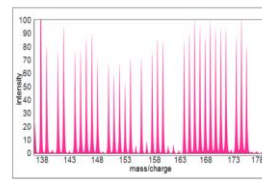
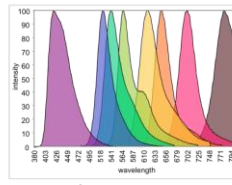


How to validate all findings from scRNA-seq?

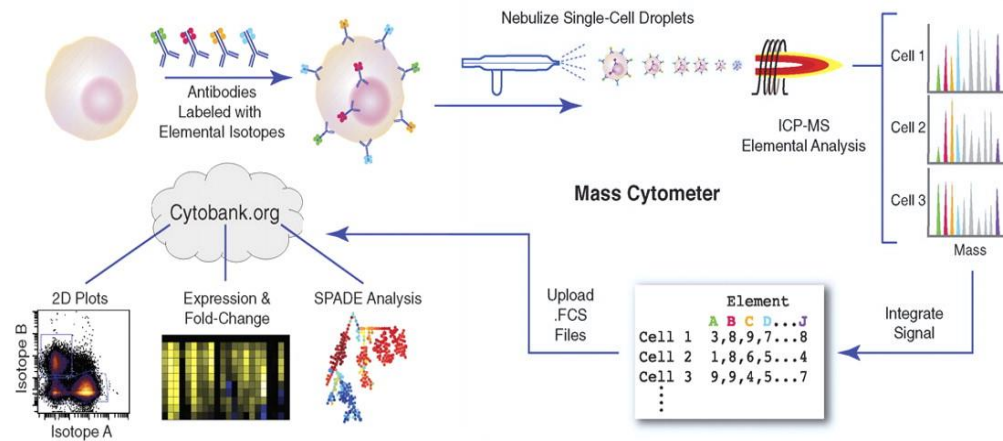


Immunophenotyping using Time Of Flight Mass Cytometry (CyTOF)

Fluorescence based cytometry is limited by spectral overlap



Mass spectrometry based cytometry permits evaluation of many more parameters



CyTOF 2



Adapted from Bendall et al, 2011

Why use both scRNA-seq and CyTOF for analyses?

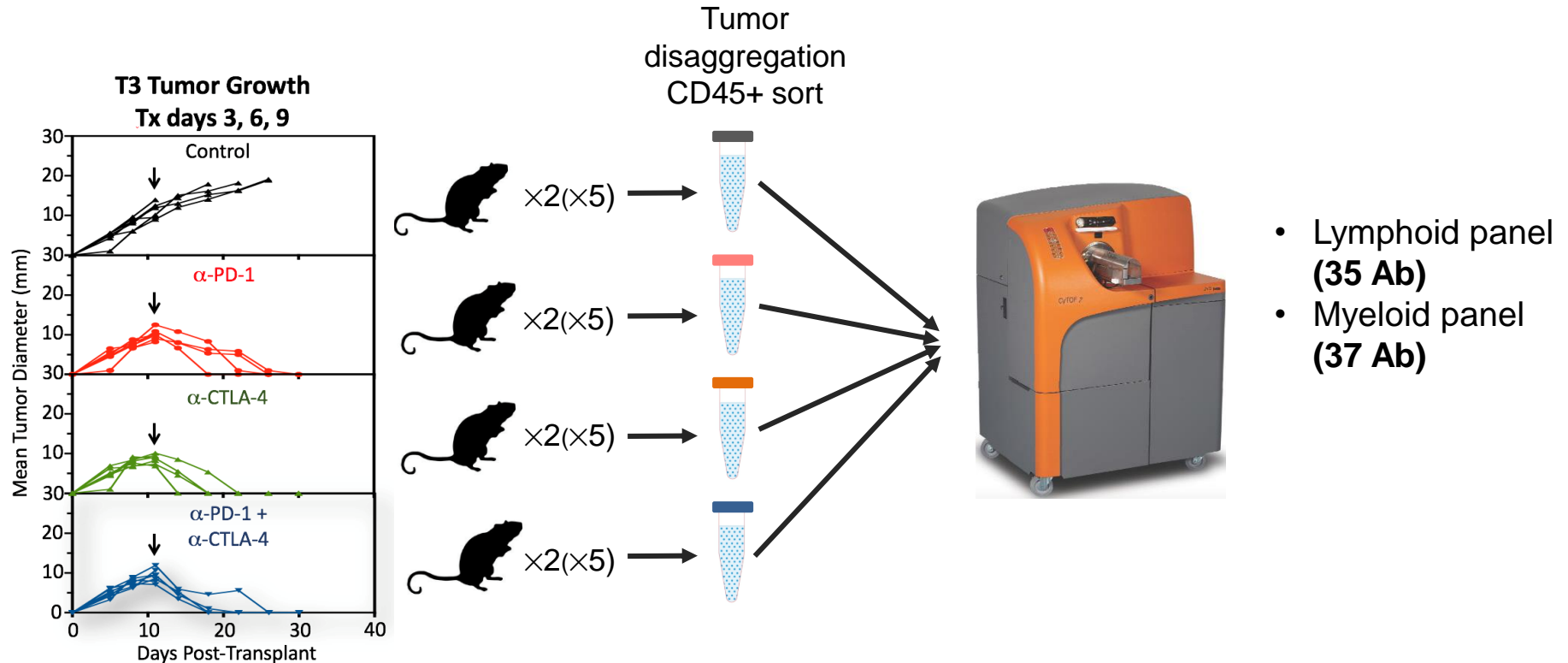
scRNA-seq

- more expensive
- detects transcripts
- ~4000 genes per cell
- ~10.000 cells per sample
- unbiased clustering

CyTOF

- reasonably affordable
- detects proteins
- ~30-40 proteins
- ~100.000 cells per sample (better to see minor cell populations)
- biased clustering

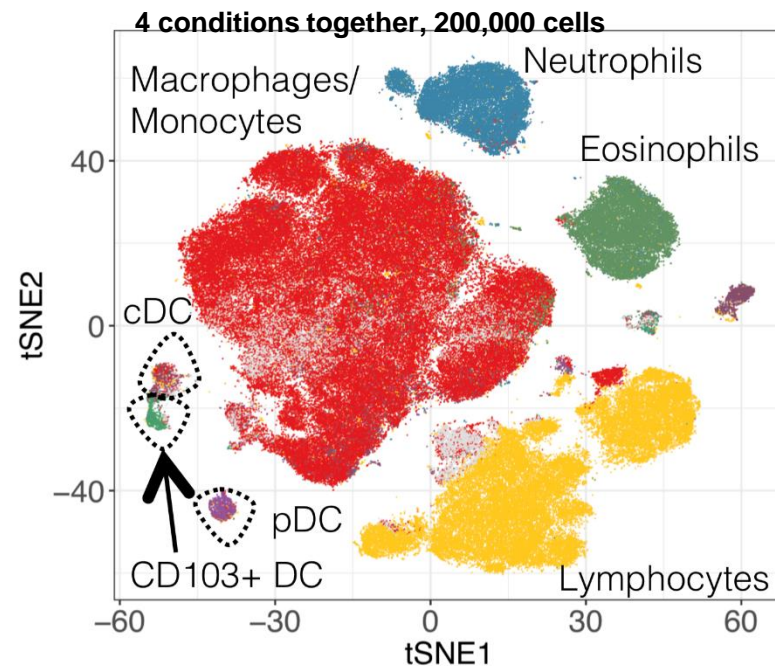
CyTOF analysis of Tumor Infiltrating Leukocytes's



Antibody panels, designed to dissect two major TIL populations

Lymphoid		Myeloid	
CD44	CD90.2 (Thy1.2)	B220	CD83
CD127 (IL-7Ra)	CXCR3	BST2/PDCA-1	CD86
CD137 (4-1BB)	EOMES	CCR7	CD90.2 (Thy1.2)
CD150 (SLAM)	FoxP3	CD103	CX3CR1
CD152 (CTLA-4)	Granzyme B	CD11b	F4/80
CD19	Ki67	CD11c	Ki67
CD24	KLRG-1	CD124 (IL-4Ra)	Ly6C
CD25	Lag3	CD19	Ly6G
CD27	Lama4 Tetramer	CD192 (CCR2)	MerTK
CD278 (ICOS)	Ly-6A/E (Sca1)	CD1d	MHC II
CD279 (PD-1)	NKp46 (NCR1)	CD206	Nos2
CD357 (GITR)	OX-40	CD24	PD-L1
CD38	T-bet	CD38	PD-L2
CD39	TCR beta	CD40-PE	Siglec F
CD4	Alg8 Tetramer	CD43	SIRPa
CD45	Tim-3	CD45	VCAM1
CD62L	VISTA	CD63	VISTA
CD8a		CD64	XCR1
		CD80	

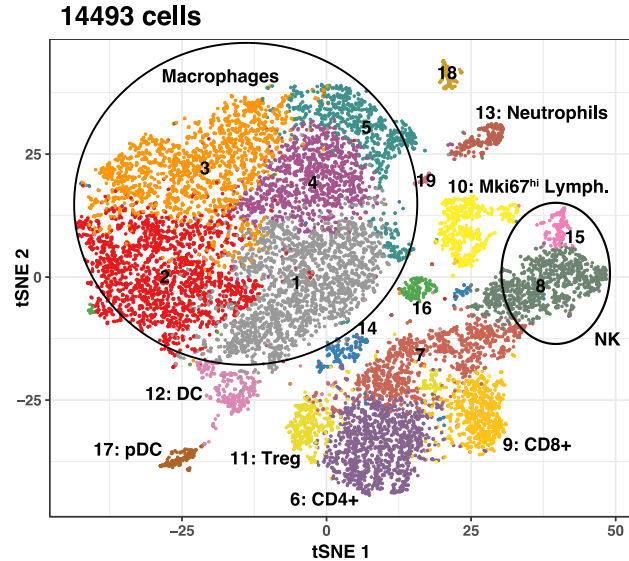
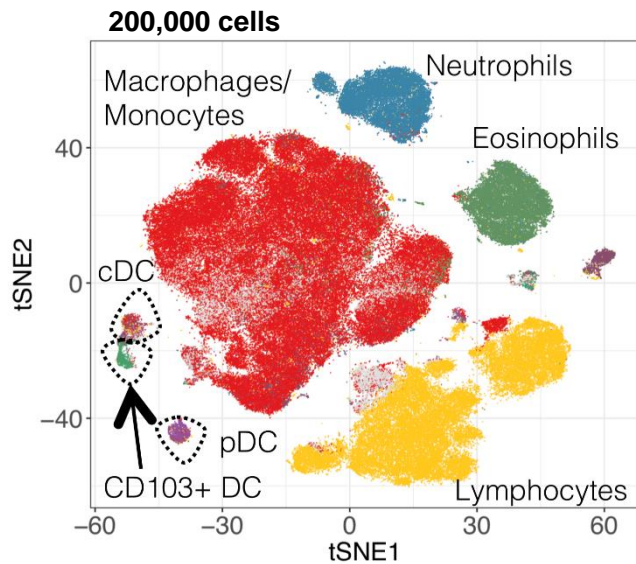
CyTOF: macrophages constitute large portion of cells



Striking correspondance between the transcriptomic and protein data

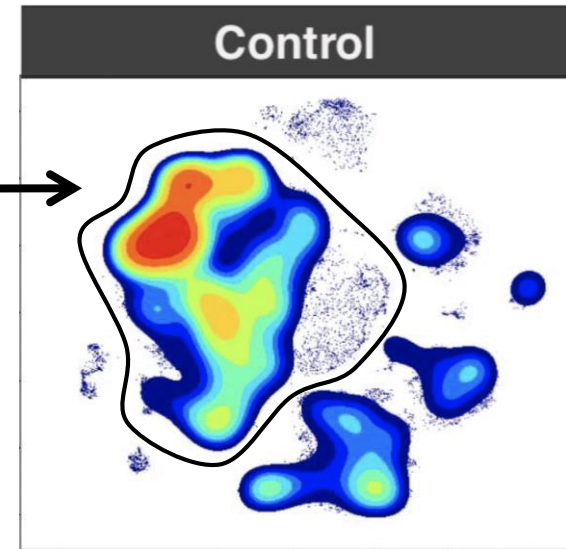
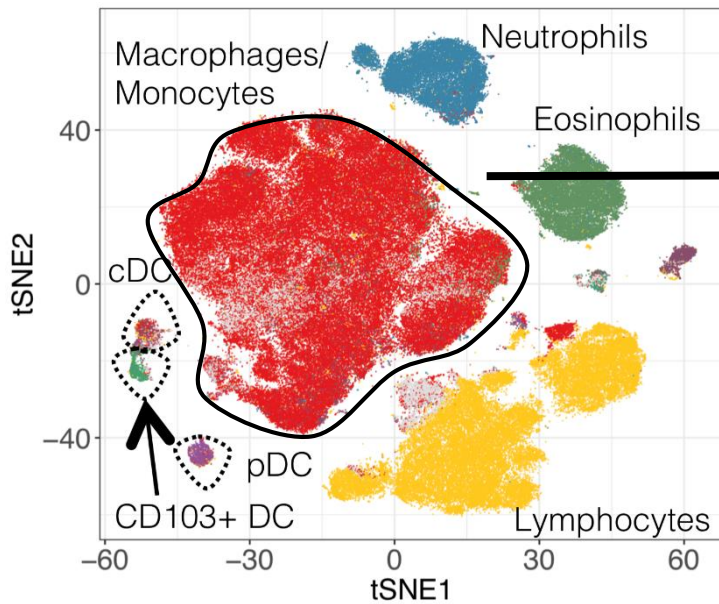
CytoTOF

scRNA-seq

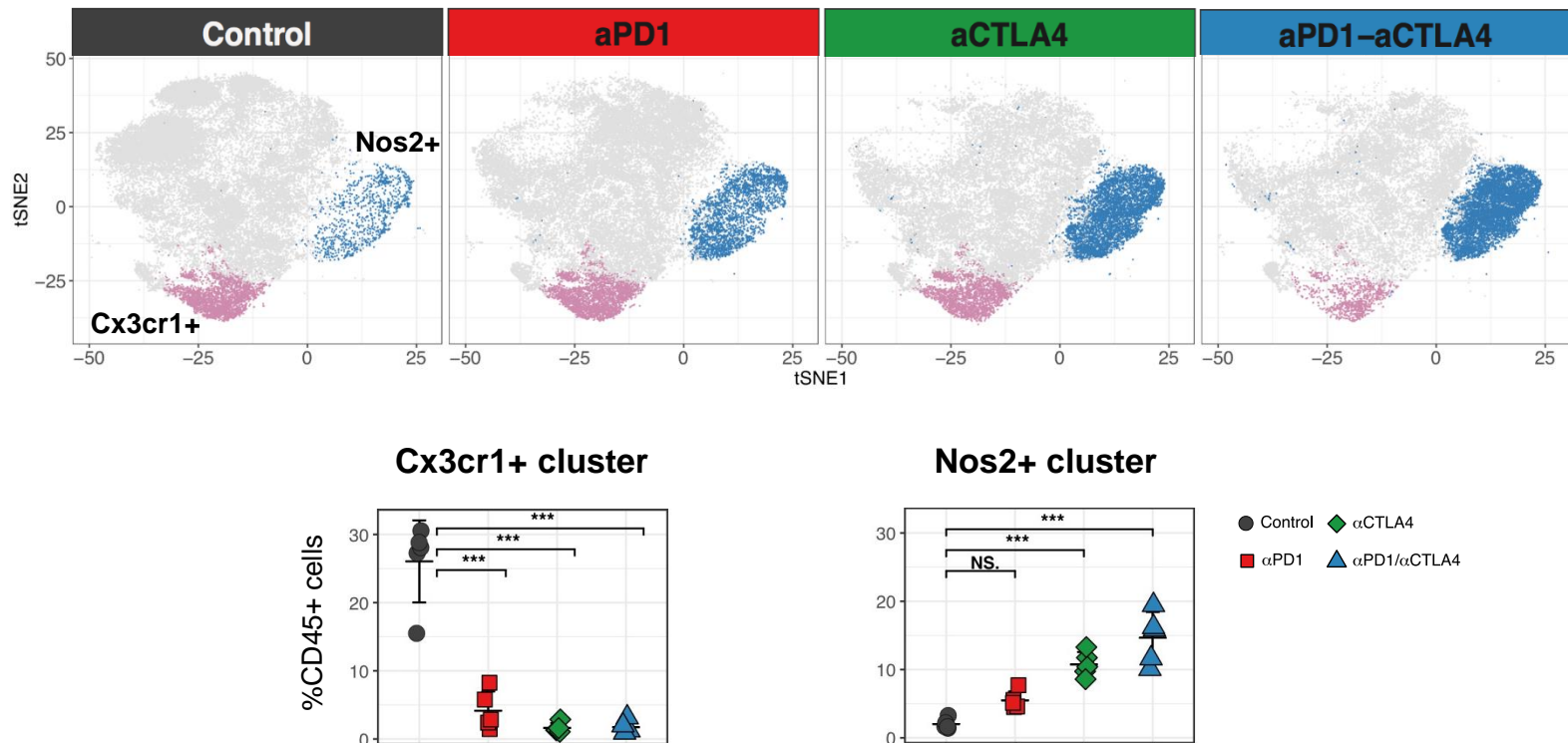


Macrophages constitute large portion of cells and undergo changes with treatments

4 conditions, 150.000 cells

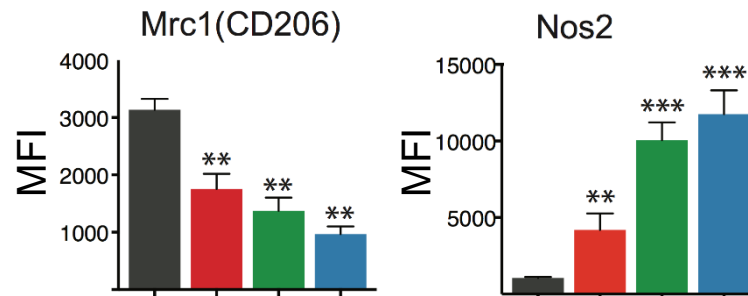
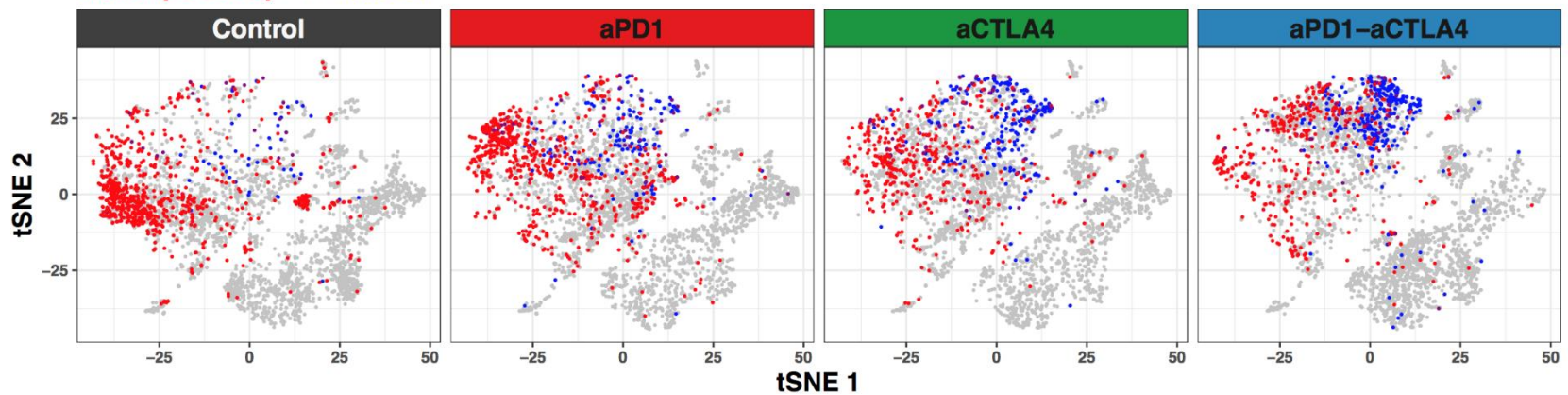


Cx3cr1 and *Nos2* correspondence between scRNA-seq data and CyTOF

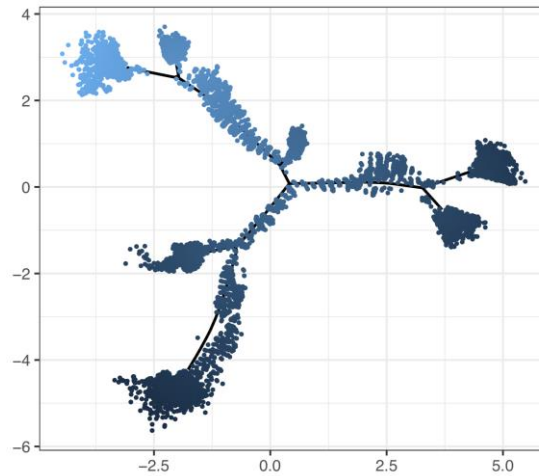
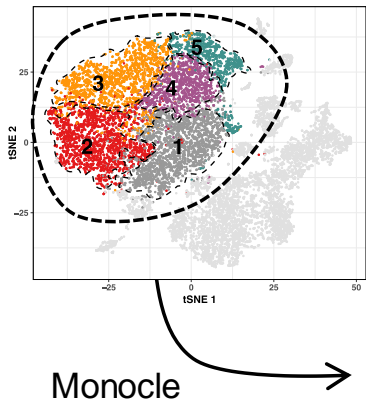


Transition from anti-inflammatory to pro-inflammatory macrophages?

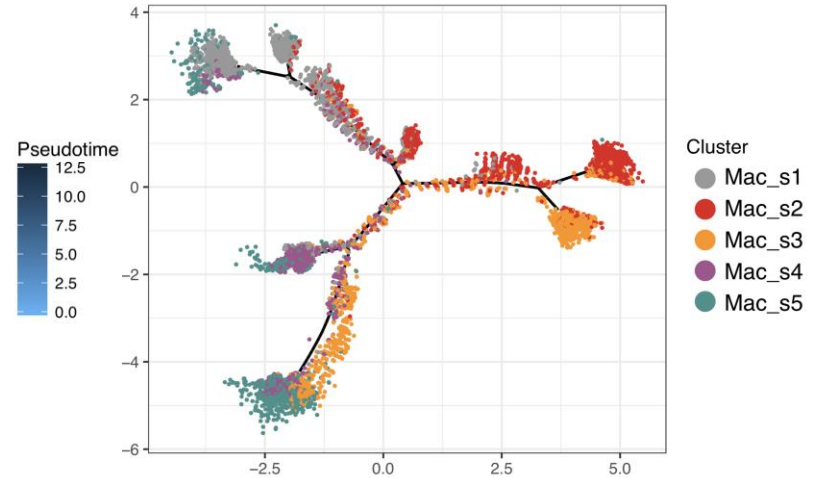
Mrc1 (CD206) and **Nos2**



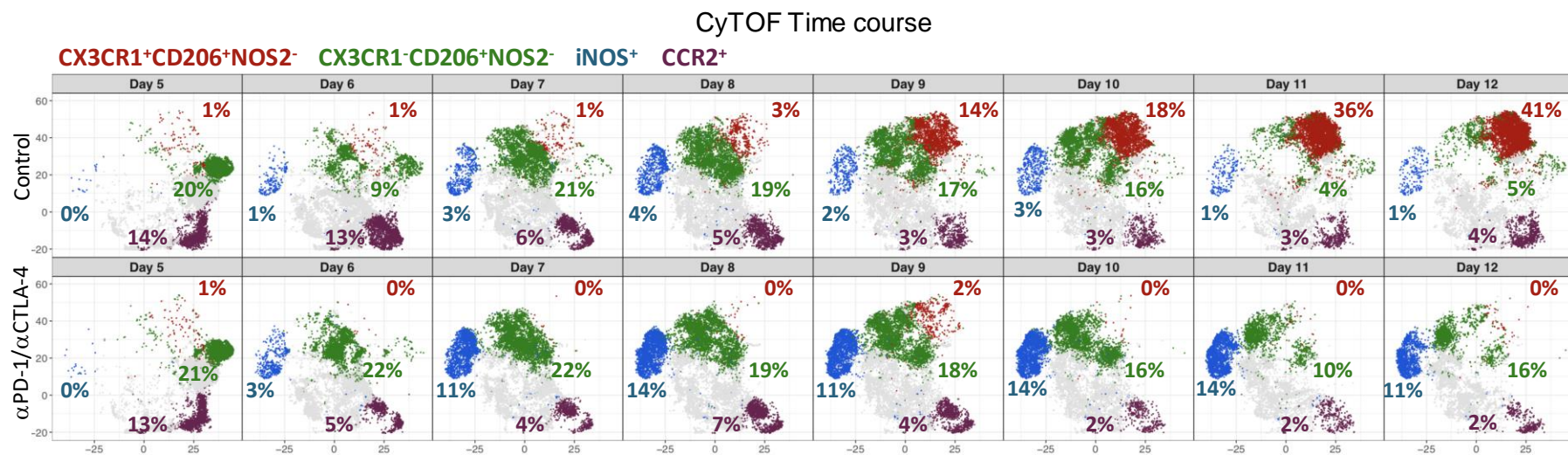
Pseudotime suggest that monocytes are the “point of origin”



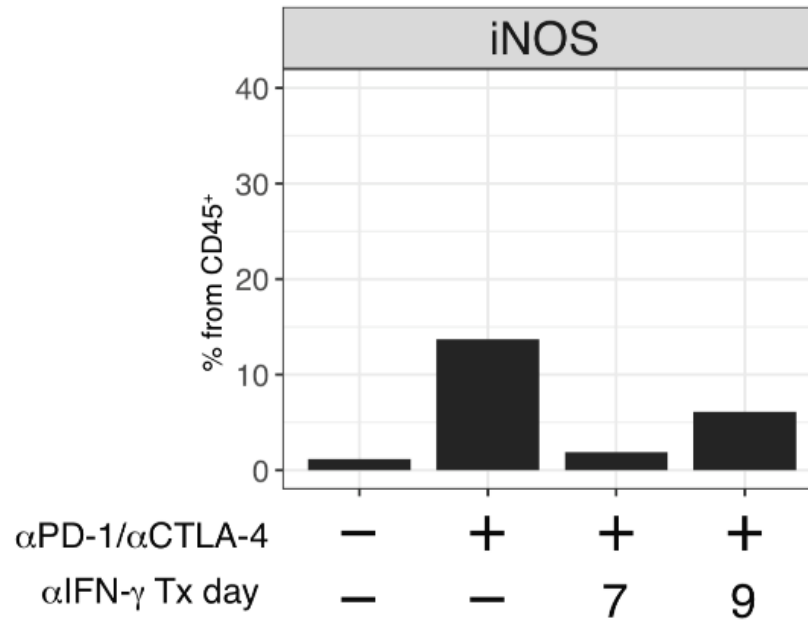
B



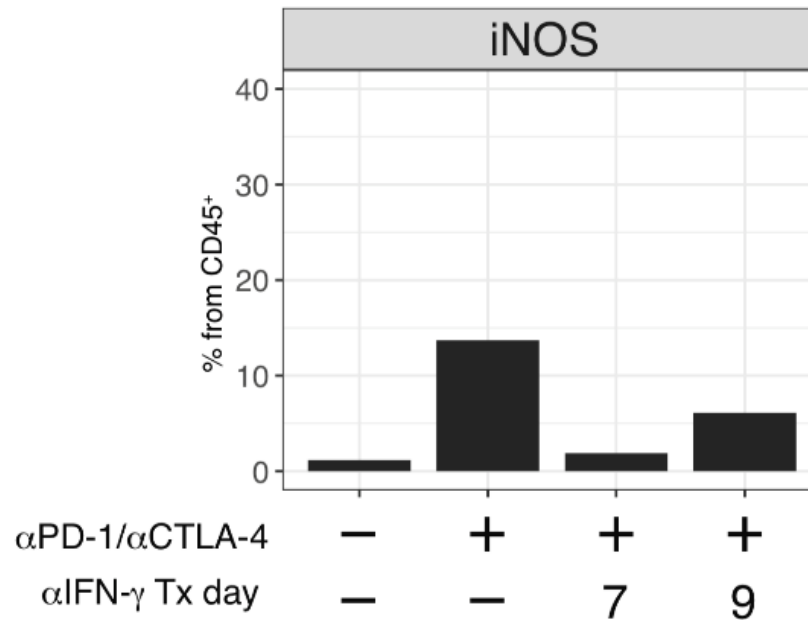
CYTOF timecourse confirms this directly



Development of proinflammatory subset is IFN γ dependent

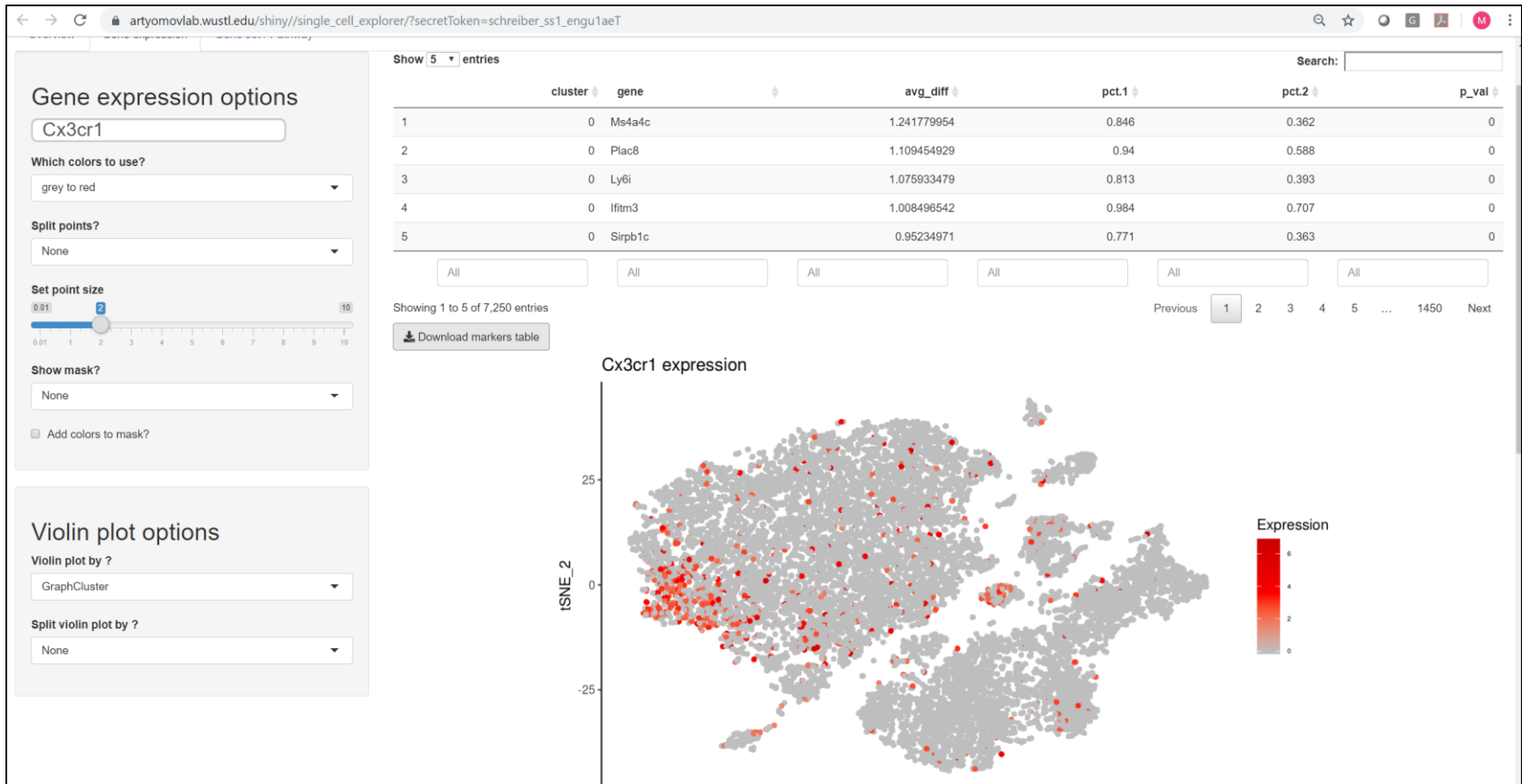


Development of proinflammatory subset is IFN γ dependent



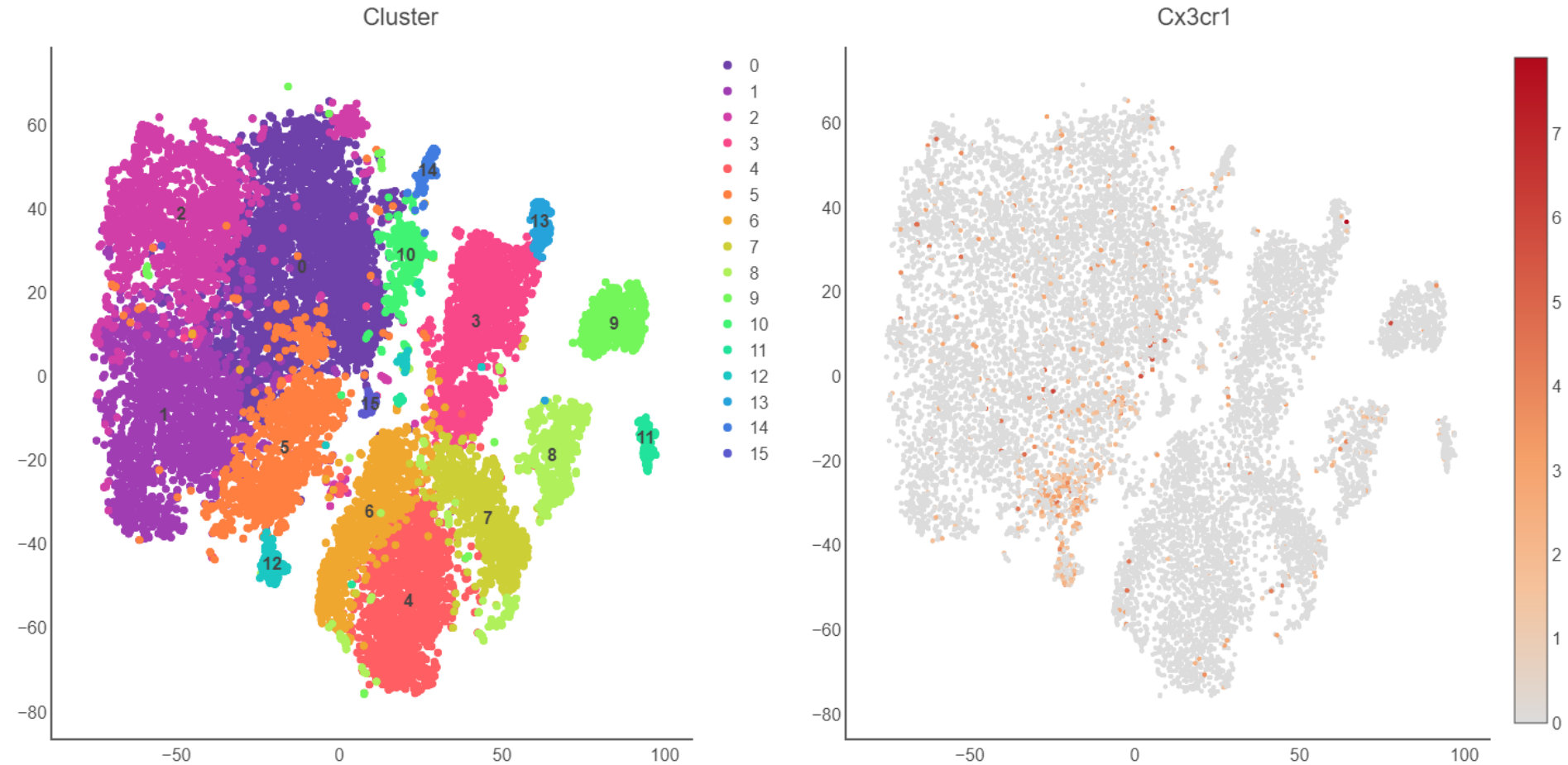
How about anti-inflammatory Cx3cr1+ macrophages?

Cluster 0 is the Cx3cr1+ population



Cluster 5 is the Cx3cr1+ population

GSE119352_SRA765288 @ <http://artyomovlab.wustl.edu/sce/>



Download Cluster 5 markers

Single-cell Explorer: Beta GSE119352_SRA765288

Overview
Histogram / Bar plot
Expression scatter plot
Expression violin plot
Pathway / Gene set plot
Markers
Files

Choose the table
Cluster

Gene name	Cluster	Av. log-fold change	P value	Adjusted p value	% in cluster	% outside
..	= 5	>	< 1e-	< 1e-	>	<
M54a7	5	1.4392	0	0	0.715	0.195
C1qc	5	1.2808	0	0	0.916	0.396
Lgmn	5	1.2697	0	0	0.957	0.407
C1qa	5	1.2217	0	0	0.916	0.402
C1qb	5	1.1951	0	0	0.938	0.471
Cd81	5	1.1828	0	0	0.561	0.088
Rgs10	5	1.0964	0	0	0.844	0.279
Tgfb1	5	1.021	0	0	0.957	0.431
Dab2	5	0.9564	0	0	0.752	0.22
Hebp1	5	0.9488	0	0	0.758	0.243

Previous Page 1 of 24 10 rows Next

Download current table

Download Cluster 5 markers

markers (2) - Excel

File Home Insert Page Layout Formulas Data Review View Tell me what you want to do... Sign in Share

Clipboard Font Alignment Number Styles Cells Editing

gene

gene	cluster	avg_logFC	p_val	p_val_adj	pct1	pct2
Ms4a7	5	1.4392	0	0	0.715	0.195
C1qc	5	1.2808	0	0	0.916	0.396
Lgmn	5	1.2697	0	0	0.957	0.407
C1qa	5	1.2217	0	0	0.916	0.402
C1qb	5	1.1951	0	0	0.938	0.471
Cd81	5	1.1828	0	0	0.561	0.088
Rgs10	5	1.0964	0	0	0.844	0.279
Tgfb1	5	1.021	0	0	0.957	0.431
Dab2	5	0.9564	0	0	0.752	0.22
Hebp1	5	0.9488	0	0	0.758	0.243
Ntpr	5	0.8569	0	0	0.51	0.105
Rnase4	5	0.7619	0	0	0.403	0.067
RP24.324L	5	0.6762	0	0	0.526	0.1
Clec4a2	5	0.9457	#####	#####	0.722	0.237
RP23.161E	5	0.5846	#####	#####	0.691	0.187
Gatm	5	0.8986	#####	#####	0.845	0.347
Msr1	5	0.8126	#####	#####	0.725	0.245
Rpl17.ps9	5	0.5656	#####	#####	0.622	0.16
Cx3cr1	5	0.5758	#####	#####	0.256	0.03
Igfbp4	5	0.6114	#####	#####	0.32	0.048
Cfp	5	0.8286	#####	#####	0.832	0.339
Rab3il1	5	0.5797	#####	#####	0.422	0.086
Ms4a6c	5	0.7952	#####	#####	0.94	0.481
H2.Eb1	5	0.7314	#####	#####	0.999	0.714
Adgre1	5	0.7421	#####	#####	0.564	0.166
H2.DMb1	5	0.7225	#####	#####	0.962	0.513
Igfb5	5	0.5054	#####	#####	0.305	0.05
Aif1	5	0.7108	#####	#####	0.953	0.484
Cd72	5	0.5614	#####	#####	0.341	0.063
Mrc1	5	0.6636	#####	#####	0.507	0.132
Ctsc	5	0.7548	#####	#####	0.975	0.635
Ms4a6d	5	0.680	#####	#####	0.942	0.496

Ready

Insert top ~100 into GeneQuery

<http://artyomovlab.wustl.edu/genequery/searcher/>

← → ↻ ▲ Not secure | artyomovlab.wustl.edu/genequery/searcher/ 🔍 ☆ 🌐 📄 📄 📄 📄

Modules	1179	Detected gene format	SYMBOL
Detected groups	15	Genes entered	100
Min log ₁₀ (adj.p.value)	-46.54	Unique entrez IDs	88
		<input type="button" value="show gene conversion table"/>	
Apply orthology		no	

Group results

#	Experiment title	Module	log ₁₀ (adj.p.value)	Overlap	GSE	GMT
1	Rhabdomyosarcoma can be initiated in activated Pax7+ Muscle Progenitor Cells	3	-46.54	58/484	GSE25098	🔗
2	Expression data from murine sarcomas	4	-46.05	57/450	GSE46836	🔗
3	Expression data from MAP3K11/GDF15 axis is a critical driver of cancer cachexia	1	-45.65	66/680	GSE68162	🔗
4	Mouse Models of Alveolar/Embryonal Rhabdomyosarcoma & Spindle Cell Sarcomas	3	-40.89	51/477	GSE22520	🔗
5	An integrated cell purification and genomics strategy reveals multiple regulators of pancreas development.	12	-39.37	36/155	GSE54374	🔗
6	Acute cellular injury responses in mouse renal ischemic reperfusion injury	4	-39.31	58/579	GSE52004	🔗
7	Expression data from oxaliplatin-treated tumors from mice pre-treated or not with antibiotics cocktail	7	-37.51	40/276	GSE51414	🔗
8	Mammalian cells acquire epigenetic hallmarks of human cancer during immortalization	4	-37.08	44/303	GSE39034	🔗
9	Targeting resistance to Smoothed antagonists by inhibiting the PI3K pathway	4	-36.14	54/755	GSE22007	🔗
10	Comparing effects of mTR and mTERT deletion on gene expression and DNA damage response: MEF	7	-36.02	40/248	GSE16430	🔗
11	Expression data from (mouse) normal lung fibroblasts and carcinoma-associated fibroblasts	6	-35.37	45/320	GSE48397	🔗
12	Gene expression in murine pancreatic tumor tissues in response to the blockade of macrophage colony-stimulating factor receptor (CSF1R) signal	4	-35.34	42/333	GSE57686	🔗
13	Transcriptome analysis of adult retina cell types.	2	-35.22	54/847	GSE33085	🔗
14	Conditional activation of B-catenin in adult mouse epidermis for 0, 1, or 7 days	6	-34.88	46/369	GSE1579	🔗
15	Expression data from NFI-C knock out embryonic fibroblasts	5	-33.97	37/242	GSE15871	🔗
16	Heterogeneity in MYC-Induced Mammary Tumors Determines Outcomes Following Loss of Myc Activity	2	-33.74	70/1614	GSE22406	🔗
17	The Nuclear Receptor Nr5a2 can replace Oct4 in the Reprogramming of Murine Somatic Cells to Pluripotent Cells	4	-33.44	42/340	GSE19023	🔗
18	Expression data from highly purified MMTV-Neu Tumor Initiating Cells (TICs) and the non-TIC CD24- fraction	3	-33.22	57/976	GSE29590	🔗
19	Cancers originate preferentially in adult tissue stem cells	18	-33.12	34/136	GSE40634	🔗

Insert top ~100 into GeneQuery

<http://artyomovlab.wustl.edu/genequery/searcher/>

← → ↻ Not secure | artyomovlab.wustl.edu/genequery/searcher/

Modules 1179 Detected gene format SYMBOL
Detected groups 15 Genes entered 100
Min log₁₀(adj.p.value) -46.54 Unique entrez IDs 88
Apply orthology no

Group results

#	Experiment title	Module	log ₁₀ (adj.p.value)	Overlap	GSE	GMT
1	Rhabdomyosarcoma can be initiated in activated Pax7+ Muscle Progenitor Cells	3	-46.54	58/484	GSE25098	🔗
2	Expression data from murine sarcomas	4	-46.05	57/450	GSE46836	🔗
3	Expression data from MAP3K11/GDF15 axis is a critical driver of cancer cachexia	1	-45.65	66/680	GSE68162	🔗
4	Mouse Models of Alveolar/Embryonal Rhabdomyosarcoma & Spindle Cell Sarcomas	3	-40.89	51/477	GSE22520	🔗
5	An integrated cell purification and genomics strategy reveals multiple regulators of pancreas development.	12	-39.37	36/155	GSE54374	🔗
6	Acute cellular injury responses in mouse renal ischemic reperfusion injury	4	-39.31	58/579	GSE52004	🔗
7	Expression data from oxaliplatin-treated tumors from mice pre-treated or not with antibiotics cocktail	7	-37.51	40/276	GSE51414	🔗
8	Mammalian cells acquire epigenetic hallmarks of human cancer during immortalization	4	-37.08	44/303	GSE39034	🔗
9	Targeting resistance to Smoothed antagonists by inhibiting the PI3K pathway	4	-36.14	54/755	GSE22007	🔗
10	Comparing effects of mTR and mTERT deletion on gene expression and DNA damage response: MEF	7	-36.02	40/248	GSE16430	🔗
11	Expression data from (mouse) normal lung fibroblasts and carcinoma-associated fibroblasts	6	-35.37	45/320	GSE48397	🔗
12	Gene expression in murine pancreatic tumor tissues in response to the blockade of macrophage colony-stimulating factor receptor (CSF1R) signal	4	-35.34	42/333	GSE57686	🔗
13	Transcriptome analysis of adult retina cell types.	2	-35.22	54/847	GSE33085	🔗
14	Conditional activation of B-catenin in adult mouse epidermis for 0, 1, or 7 days	6	-34.88	46/369	GSE1579	🔗
15	Expression data from NFI-C knock out embryonic fibroblasts	5	-33.97	37/242	GSE15871	🔗
16	Heterogeneity in MYC-Induced Mammary Tumors Determines Outcomes Following Loss of Myc Activity	2	-33.74	70/1614	GSE22406	🔗
17	The Nuclear Receptor Nr5a2 can replace Oct4 in the Reprogramming of Murine Somatic Cells to Pluripotent Cells	4	-33.44	42/340	GSE19023	🔗
18	Expression data from highly purified MMTV-Neu Tumor Initiating Cells (TICs) and the non-TIC CD24- fraction	3	-33.22	57/976	GSE29590	🔗
19	Cancers originate preferentially in adult tissue stem cells	18	-33.12	34/136	GSE40634	🔗

GeneQuery suggests that there is connection b/w Cx3cr1+ macrophage subset and CSF1-dependent macrophages

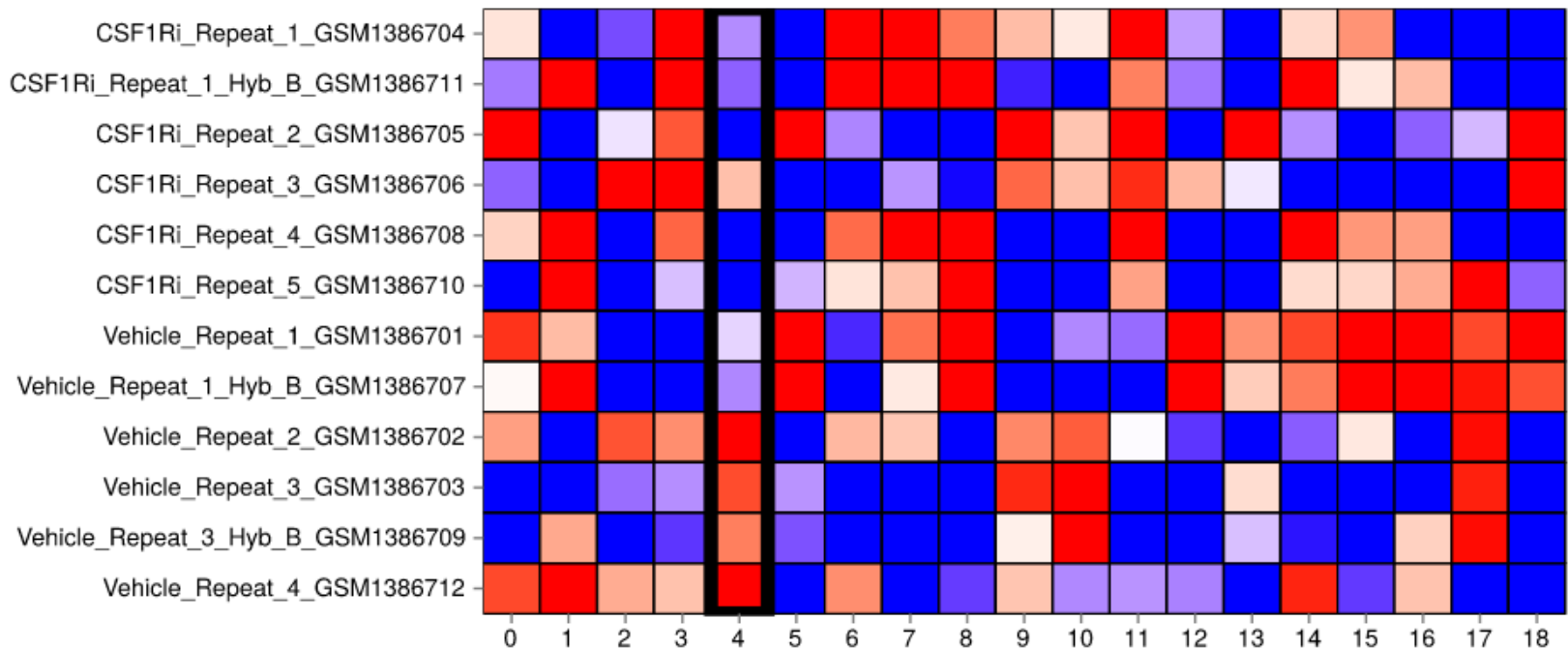
Series GSE57686

Query DataSets for GSE57686

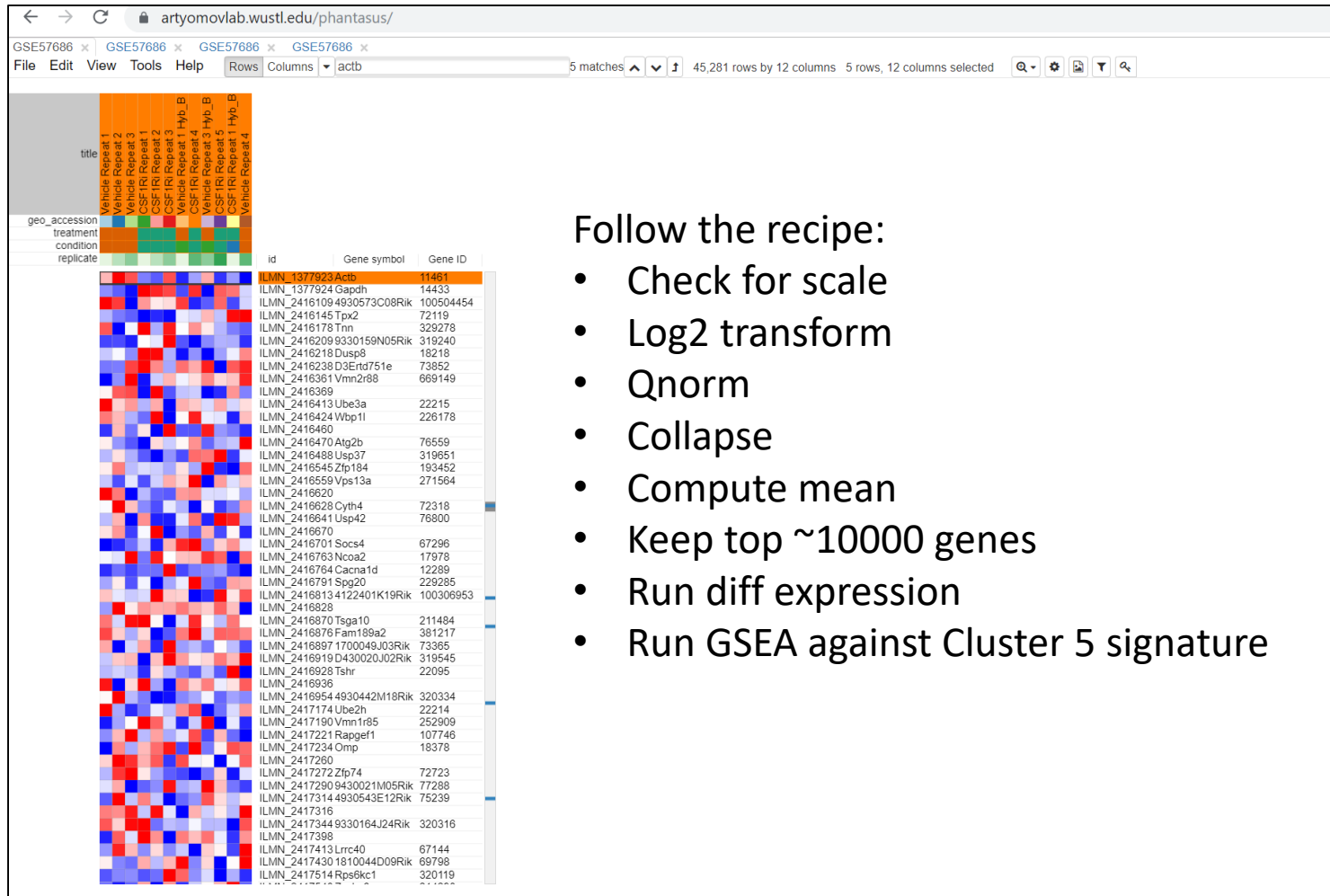
Status Public on May 15, 2014

Title Gene expression in murine pancreatic tumor tissues in response to the blockade of macrophage colony-stimulating factor receptor (CSF1R) signal

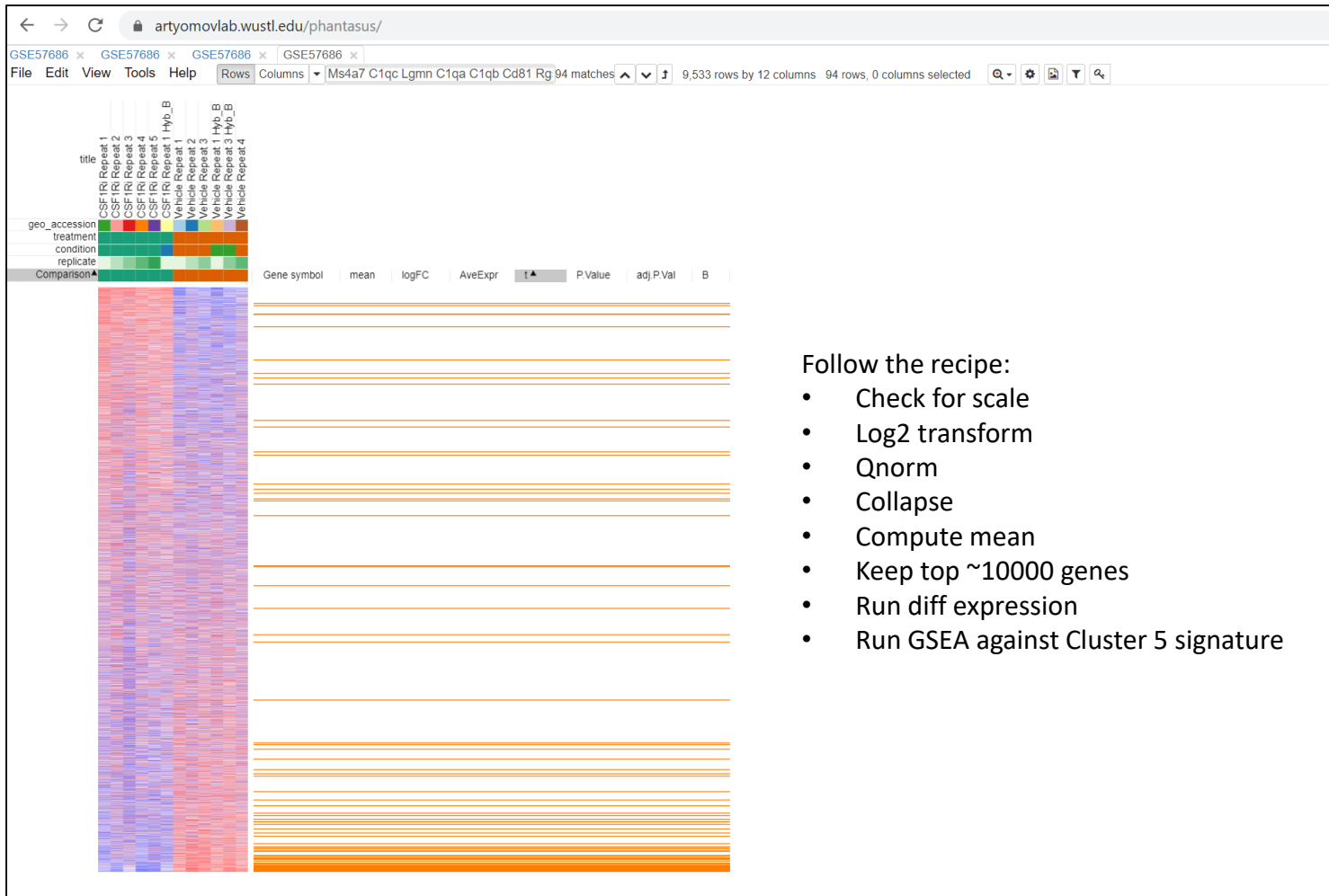
Organism *Mus musculus*



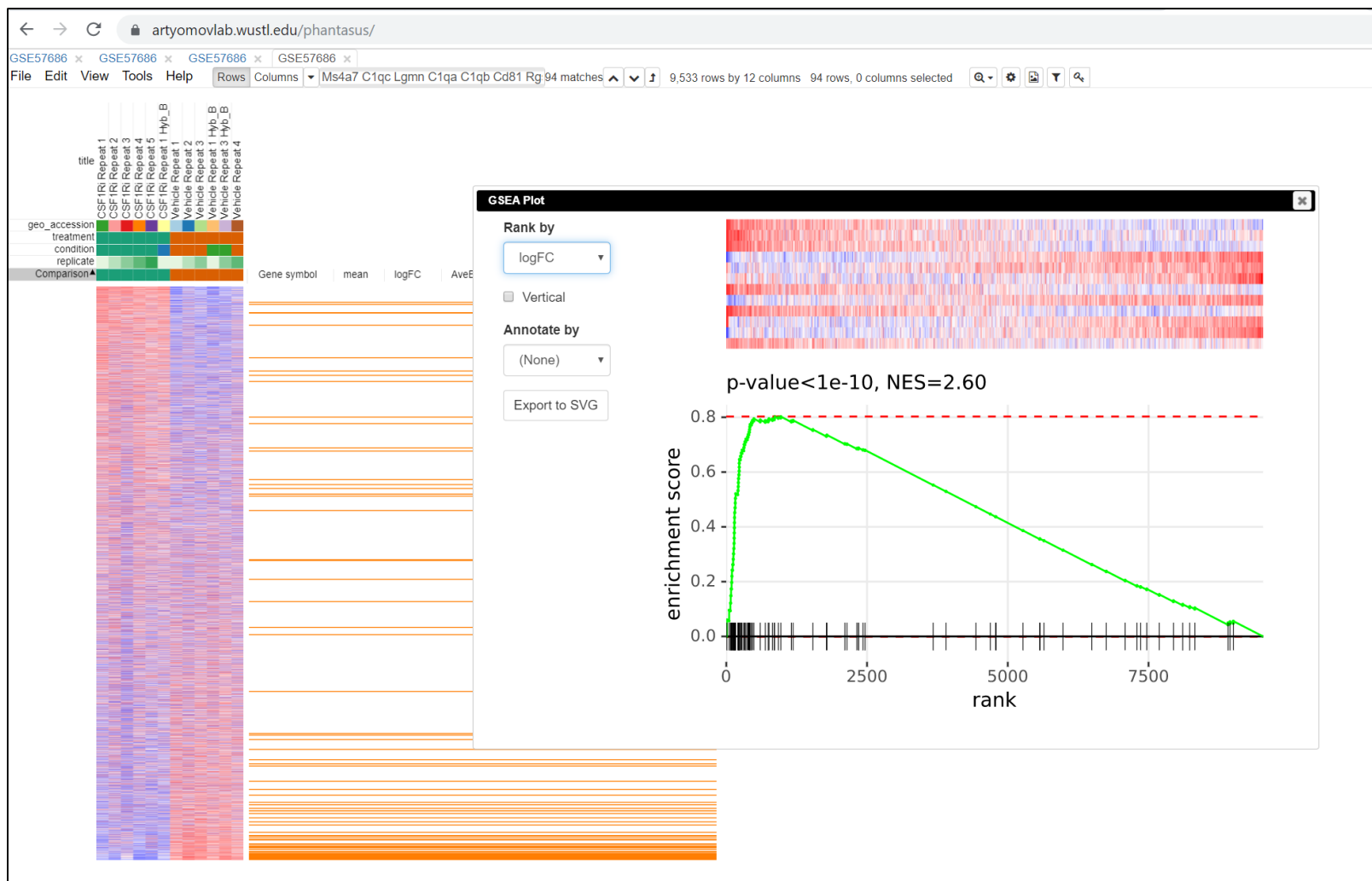
Let's go to phantastus to explore it further!



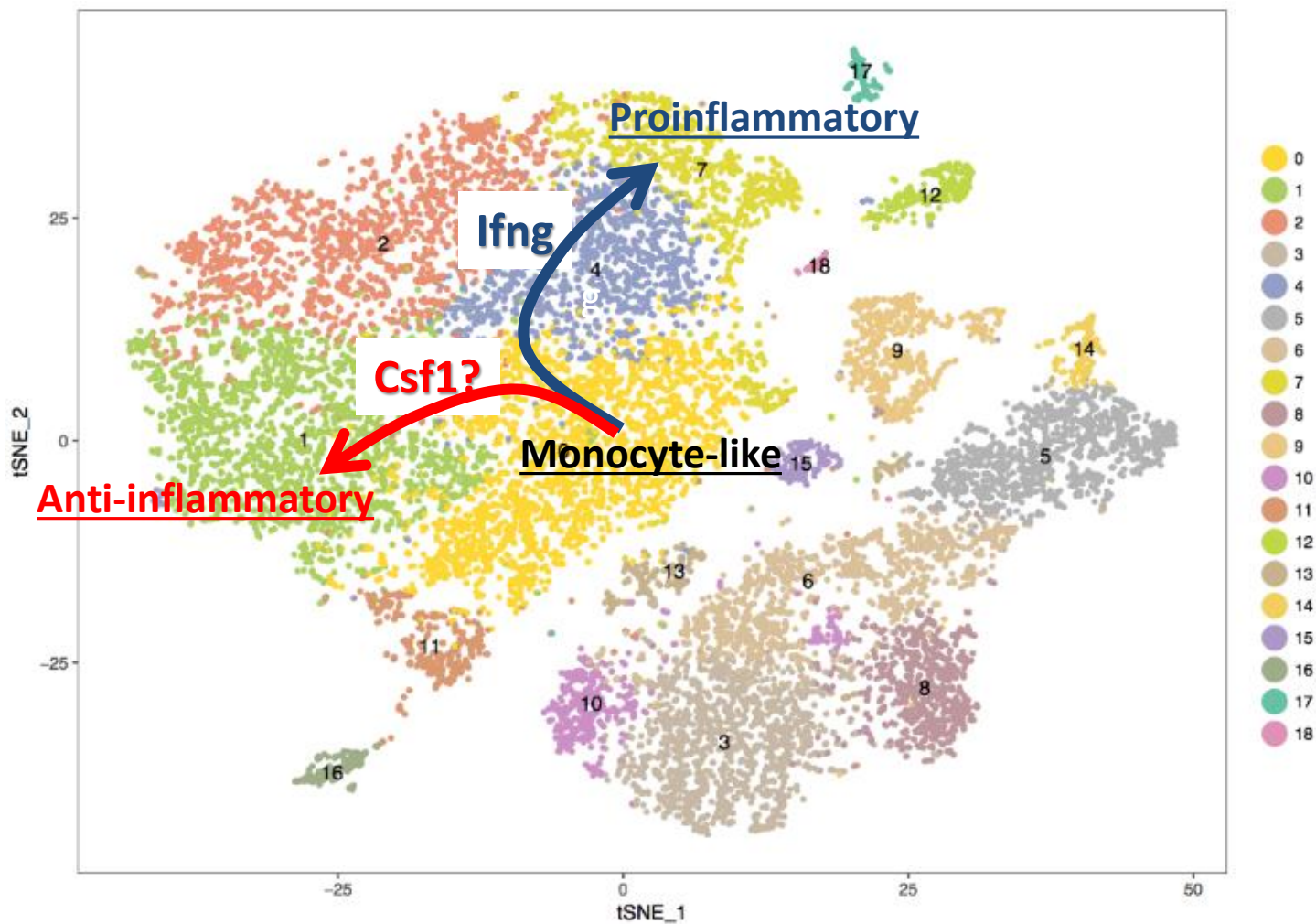
After last step..



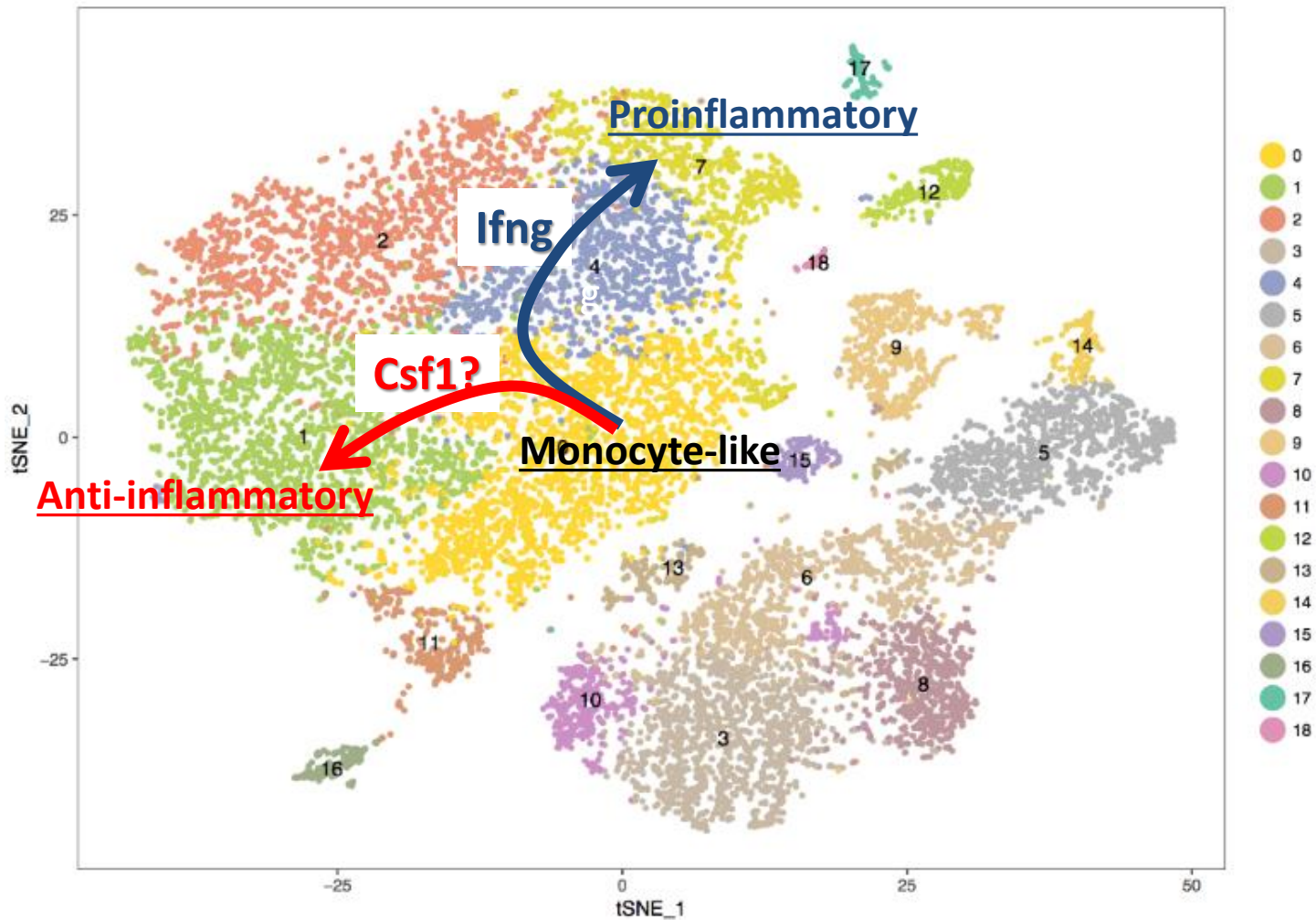
Very significant similarity: Cluster 5 predicted to be Csf1-dependent!



Ifng- vs Csf1- dependent subsets



Ifng- vs Csf1- dependent subsets



Can this knowledge help?

Combination treatments!

