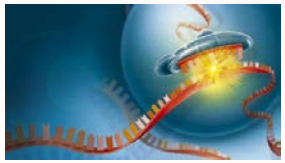
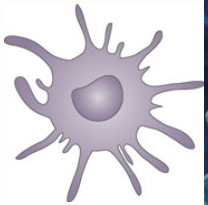
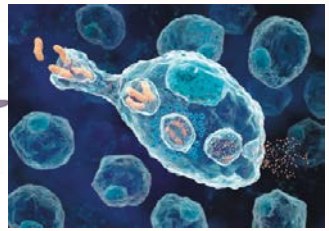
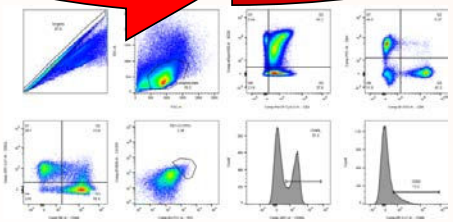
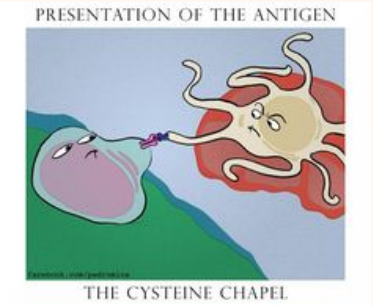
Team : IALTA
Under the supervision of Pr Gilles Foucras
UMR INRAE ENVT 1225 – IHAP

REIDSOCS project: Description of lactating mammary gland immune cells using single-cell RNA-Seq approach.



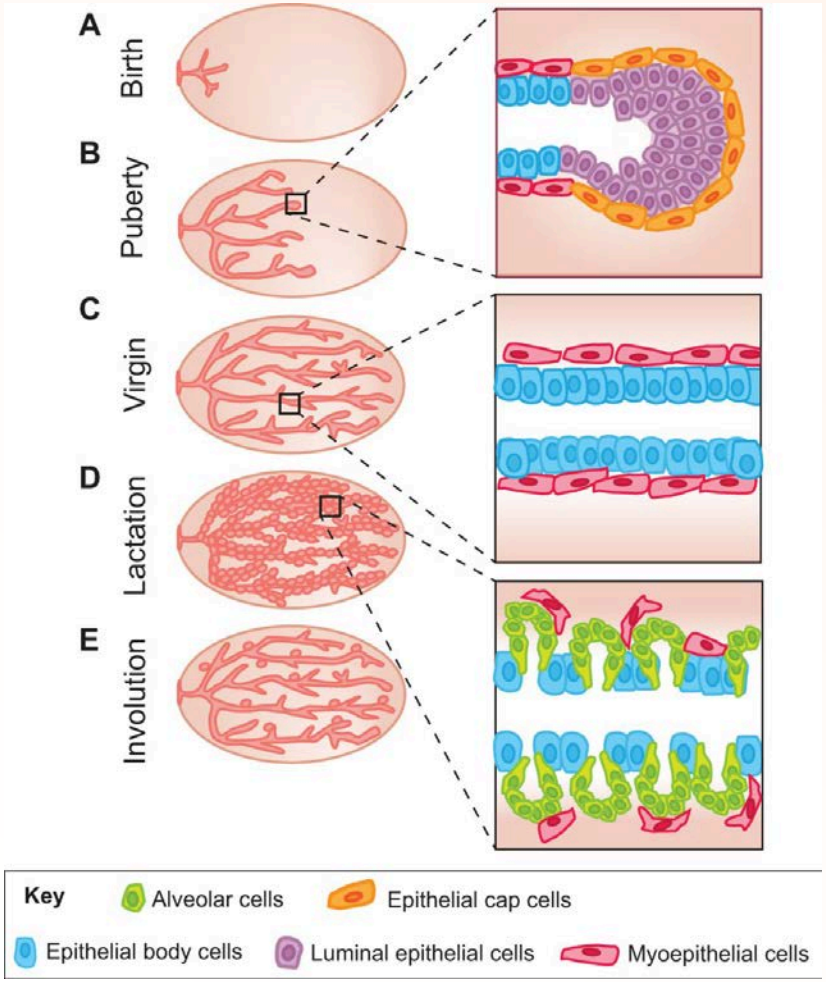



Blandine Gausseres
Laurence Guzylack
Chervin Hassel

Problematic of REIDSOCS project: milk production

Mammary gland:

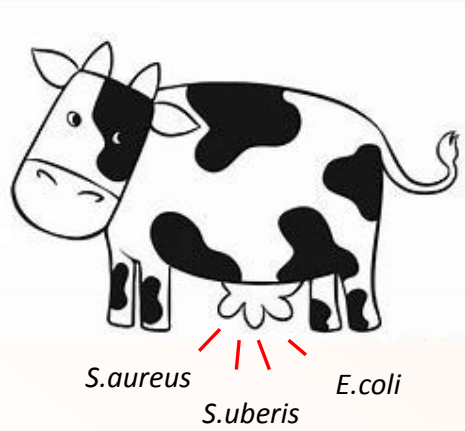


Milk : Positive trade balance of 3.5 billions €

Milk quality decreases

→ **300 000 somatic cells (CCS)/mL**

EC 2004/853 standard, April 29th 2004 → **400 000 cells/mL**

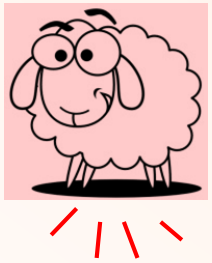


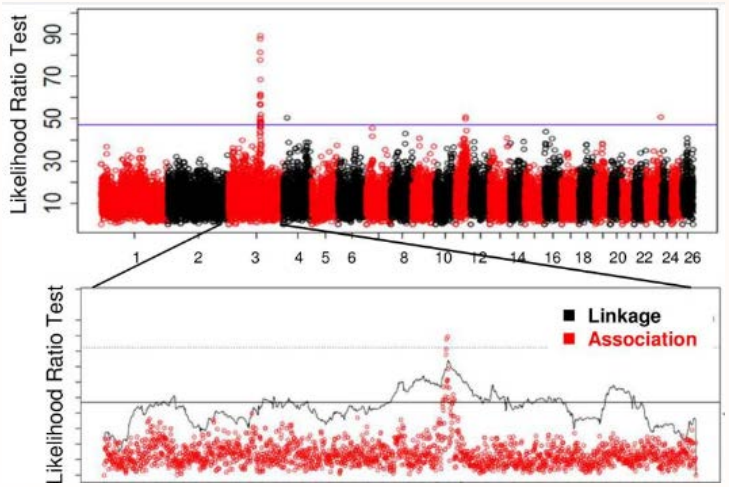
→ **Mastitis**: mammary gland infections

Problematic of REIDSOCS project: ewe genetic mutation

Resistant

Susceptible



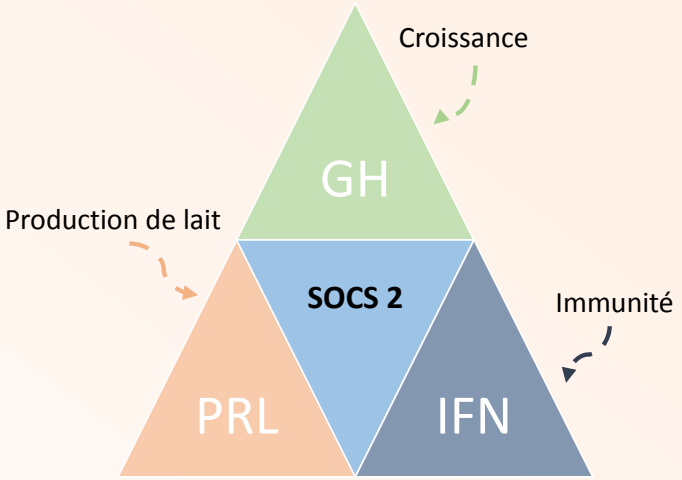


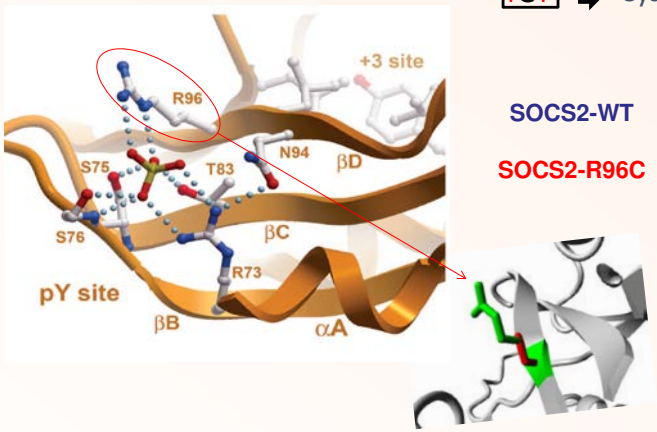
SNP SOCS-2

(suppressor of cytokine signaling 2)

Rupp Et.al 2015

SOCS-2: negative regulator of multiple signaling pathways:





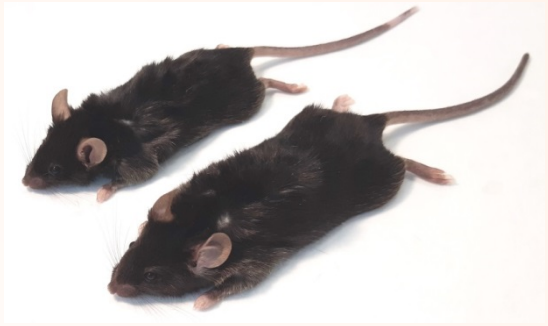
Non functionnal SOCS-2 protein

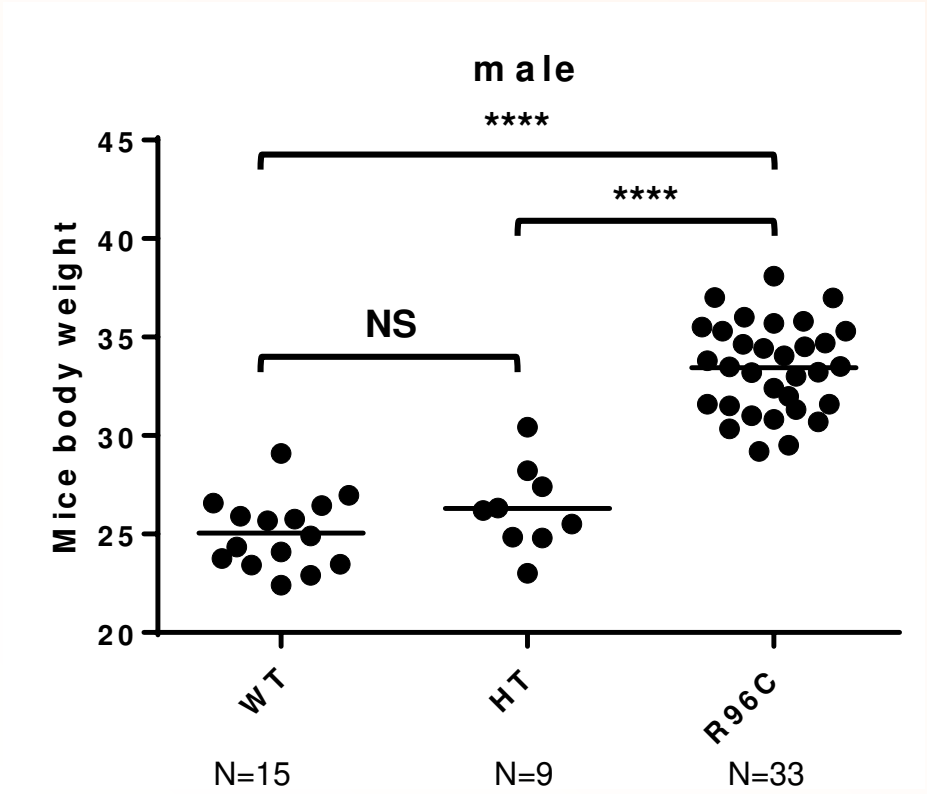
Bullock AN, […], Knapp S, *PNAS* 2006

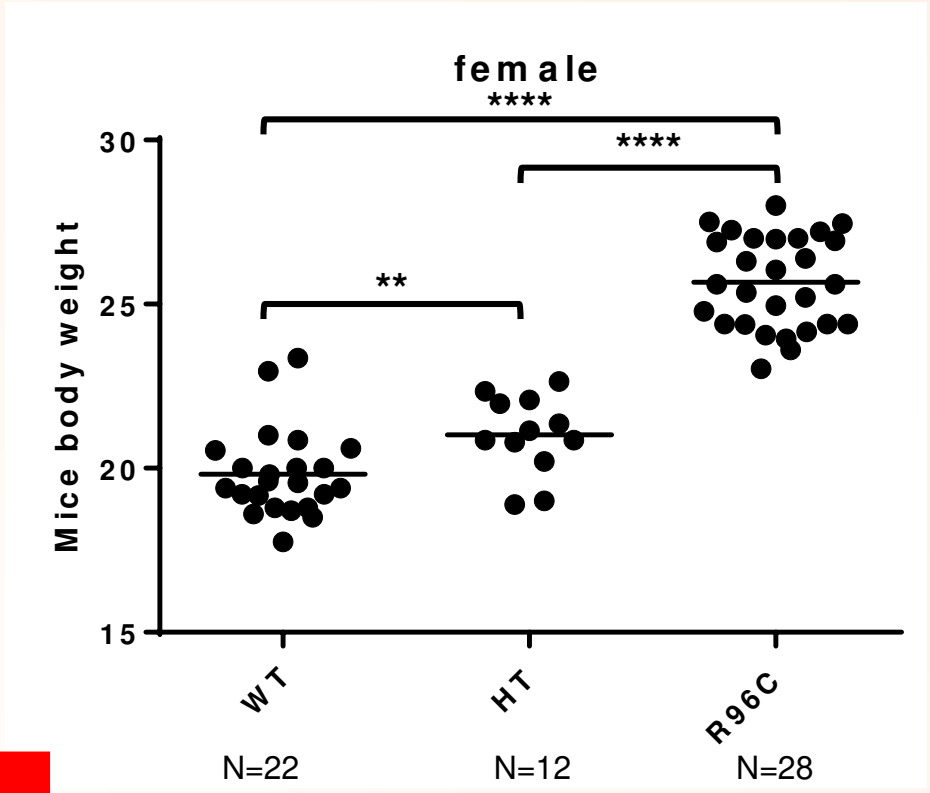
Aims of the REIDSOCS project

- Determine the immune cells composition of lactating mammary gland using unprior approach: scRNA-Seq
- Compare the immune cells composition between WT and SOCS-2 R96C KI mice using flow cytometry and scRNA-Seq
- Identify the immune function alteration during Staphylococcus aureus infection associated with SOCS-2 R96C phenotype

SOCS2^{R96C} mice

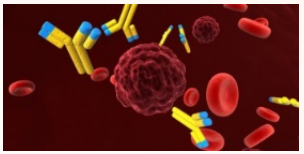




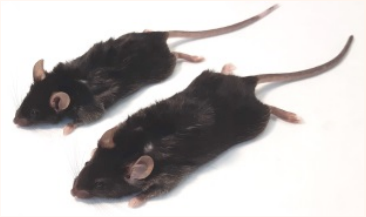


SOCS-2 invalidation: phenotype confirmation (gigantism)

Mammary gland immune cells transcriptome by scRNA-Seq

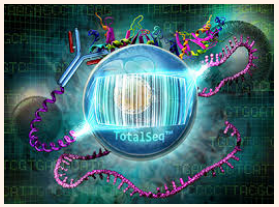


Systemic injection of CD45 antibody

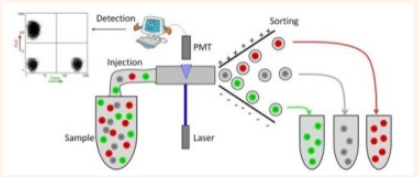


SOCS2^{R96C} vs WT mice (n = 3)

Lactating mammary gland (without LN) cells extraction



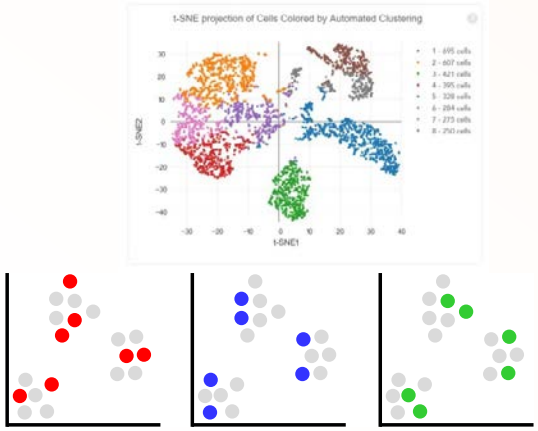
Multiplexing using CD45 TotalSeq antibody (biolegend)



FACS sort of CD45+ viable cells

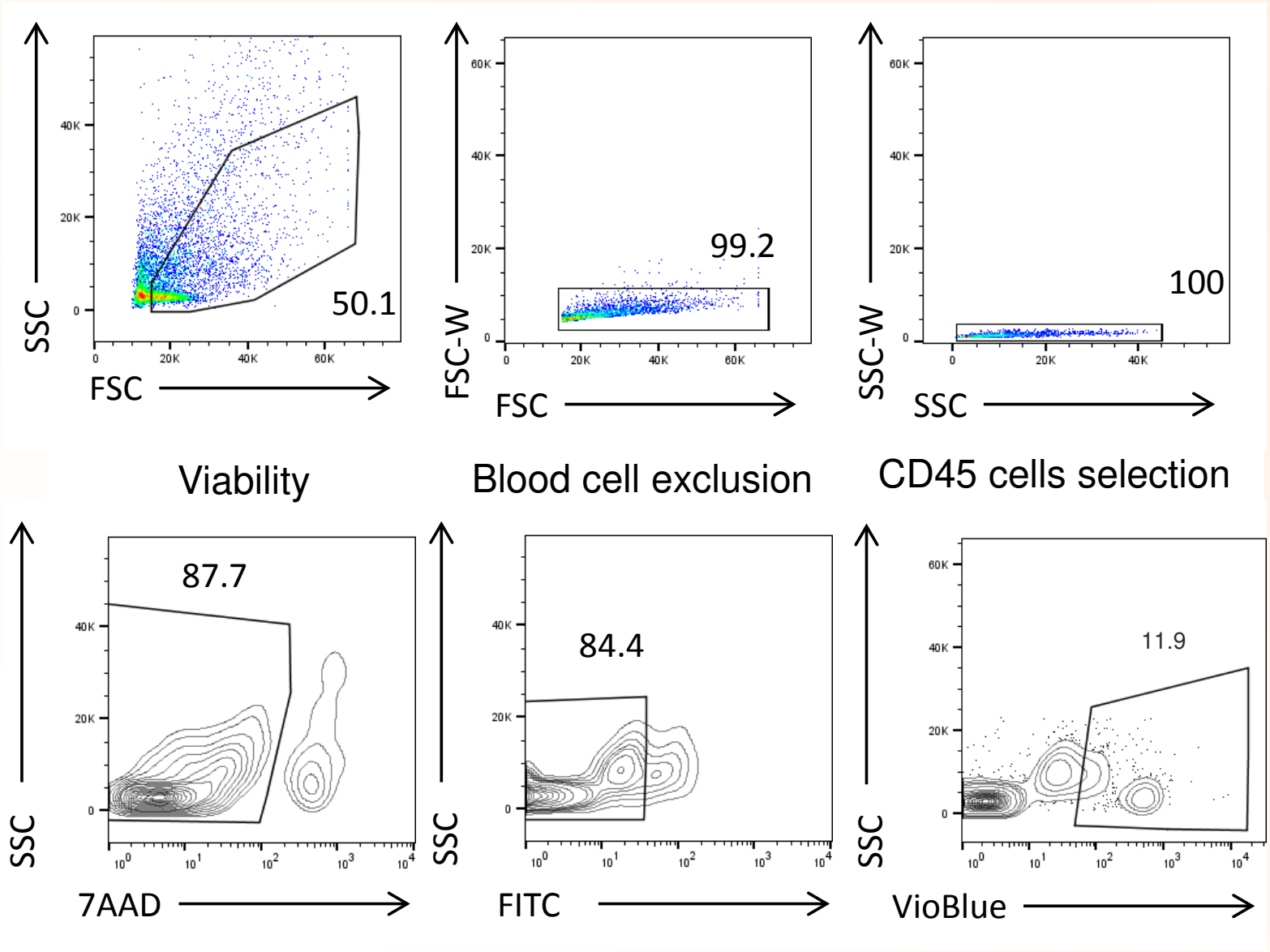
scRNA-SEQ libraries (10X genomics) + NGS Illumina sequencing

Data analysis



- **Cellular composition**
- **Gene expression levels**

1) FACS sort

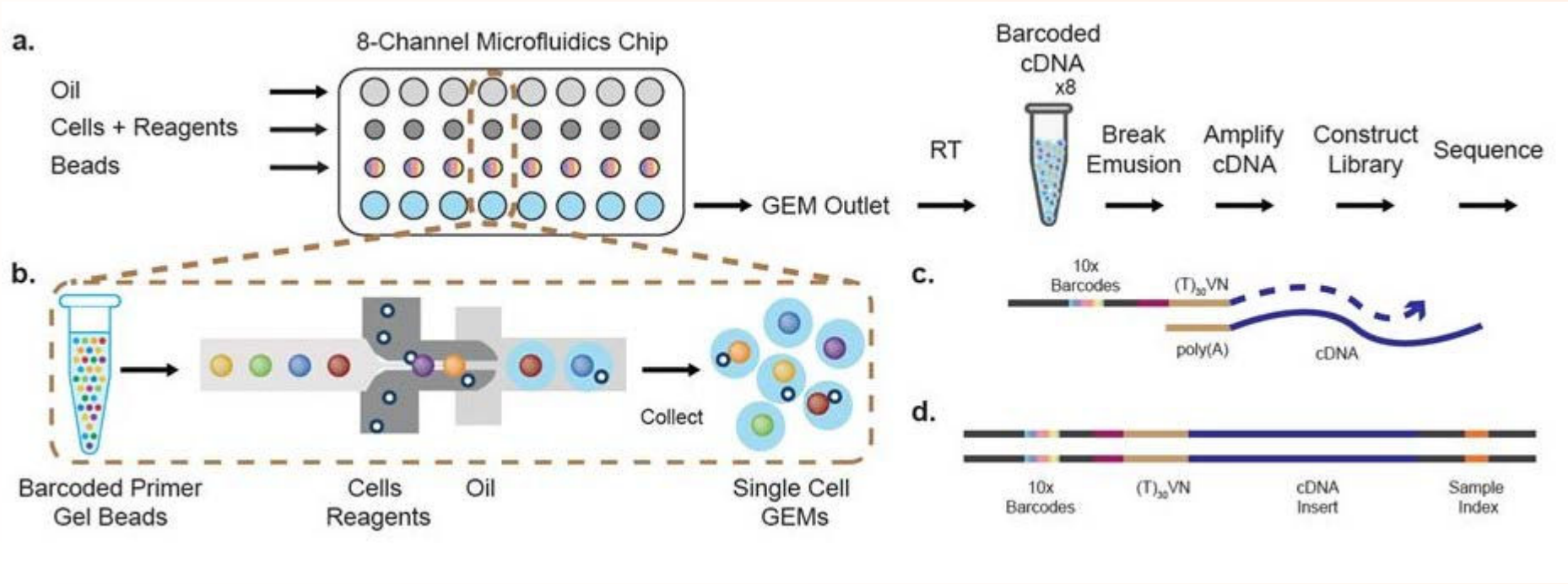


Thanks to Alexia Zakaroff-Girard and Elodie Riant

2) Librairies preparation

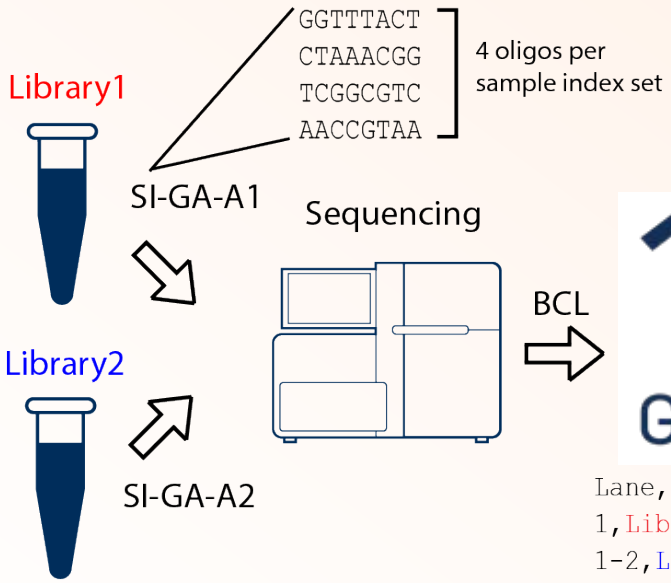
We inject 18000 cells > almost **6000 sequencing cells**

(catch rate of 33%) in 2 injection wells.



Thanks to Frederic Martins

3) Analysis pipeline of scRNASEQ data analysis



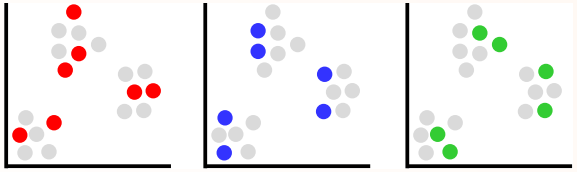
BCL

Cell ranger

10x GENOMICS

```
Lane,Sample,Index
1,Library1,SI-GA-A1
1-2,Library2,SI-GA-A2
```

CITE-seq-Count
(HTO libraries)



- TotalSeq demultiplexing
- Normalization
- Clustering and UMAP
- Cell type attribution
- Clusters

MONOCLE 3

- Trajectory inferrence

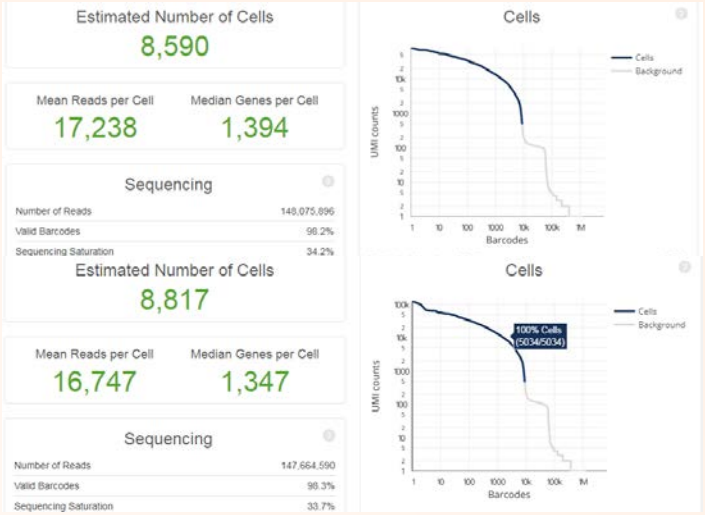
https://hoohm.github.io/CITE-seq-Count/

https://satijalab.org/seurat/

https://cole-trapnell-lab.github.io/monocle3/

Lane A Cellranger results

Lane B Cellranger results



Aggregation of A & B lanes

Estimated Number of Cells
17,407

Post-Normalization Mean Reads per Cell
16,744

Median Genes per Cell
1,360

Cells

Estimated Number of Cells	17,407
Fraction Reads in Cells	88.6%
Pre-Normalization Mean Reads per Cell	16,989
Post-Normalization Mean Reads per Cell	16,744
Median Genes per Cell	1,360
Median UMI Counts per Cell	4,232

Sequencing

Pre-Normalization Number of Reads	295,740,486
Post-Normalization Number of Reads	291,469,620

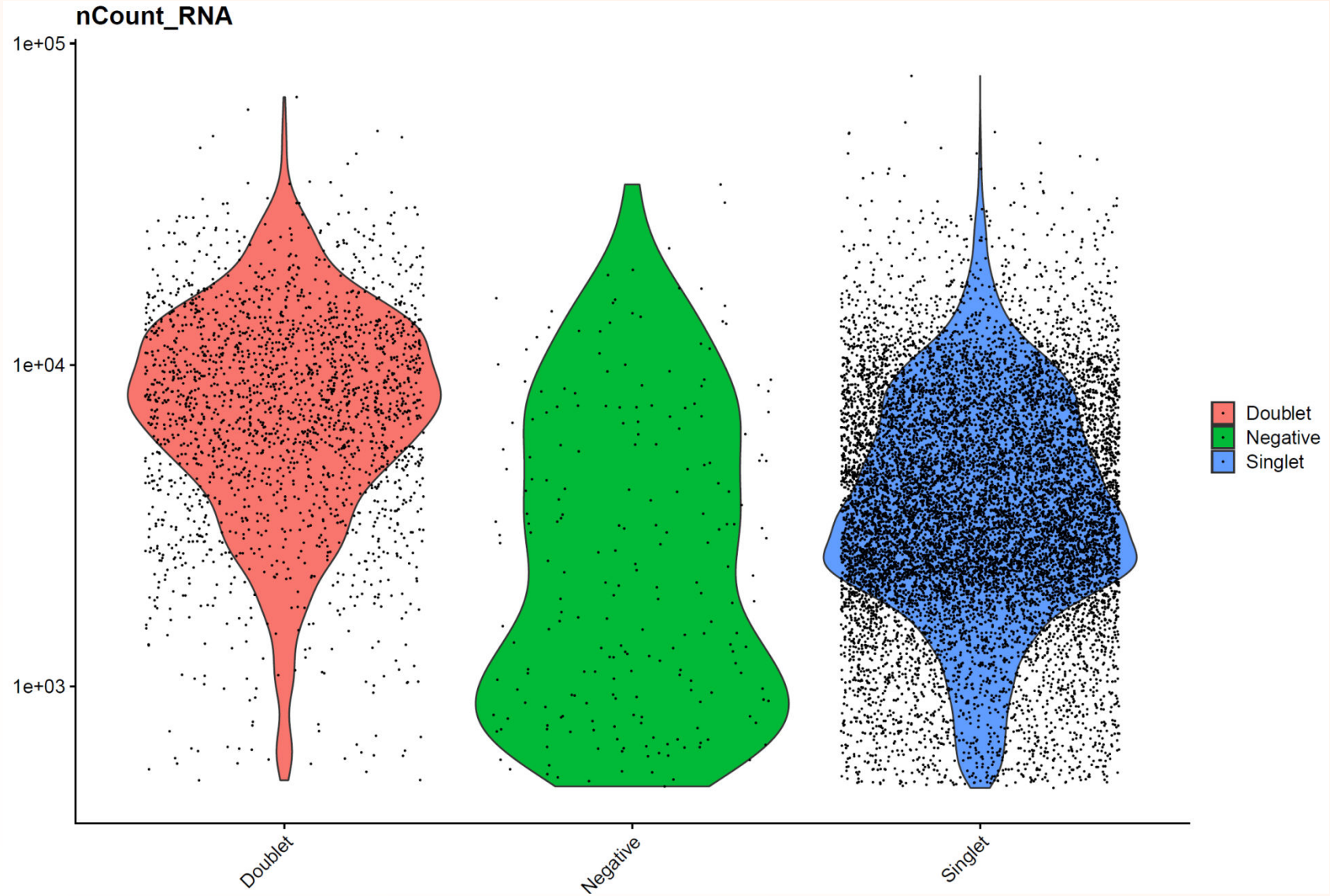
Sample

Name	SOCS
Description	
Transcriptome	mm10
Chemistry	Single Cell 3' v3
Cell Ranger Version	3.0.2

Aggregation

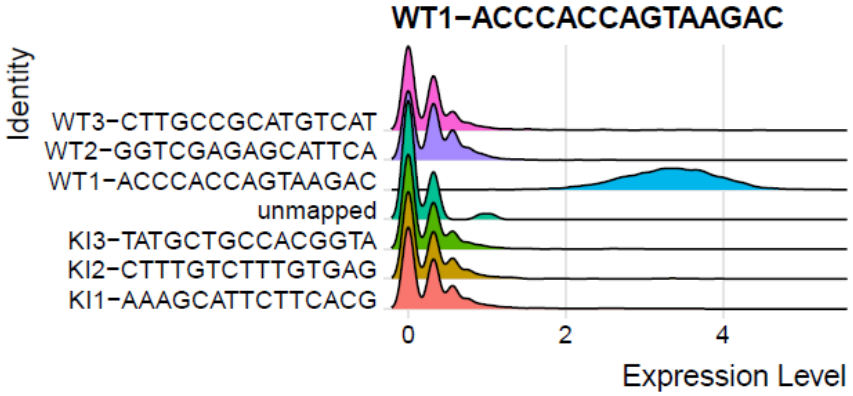
SOCS1 Fraction of Reads Kept	97.1%
SOCS2 Fraction of Reads Kept	100.0%
SOCS1 Pre-Normalization Total Reads per Cell	17,238
SOCS2 Pre-Normalization Total Reads per Cell	16,747
SOCS1 Pre-Normalization Confidently Mapped Barcoded Reads per Cell	10,135
SOCS2 Pre-Normalization Confidently Mapped Barcoded Reads per Cell	9,843

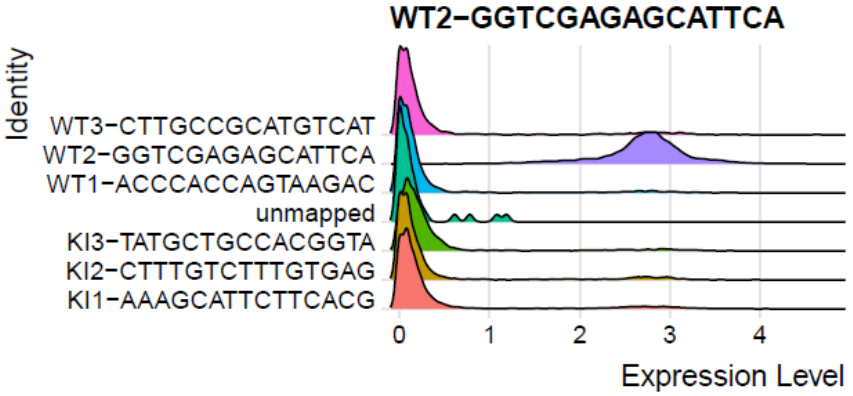
Demultiplexing with hashtag oligos and doublet exclusion under Seurat

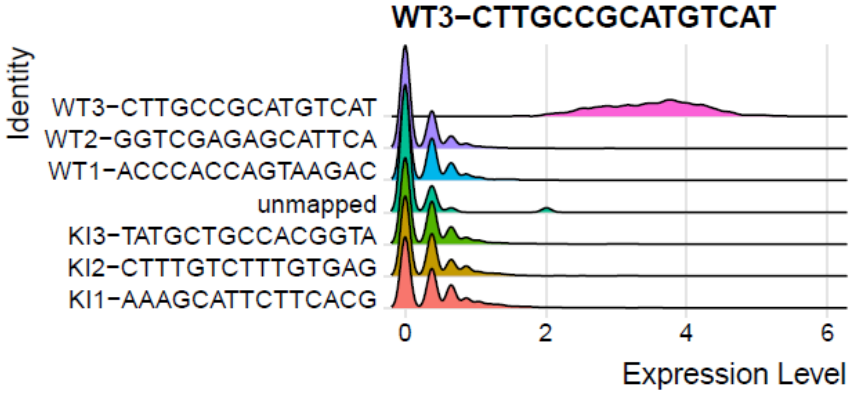


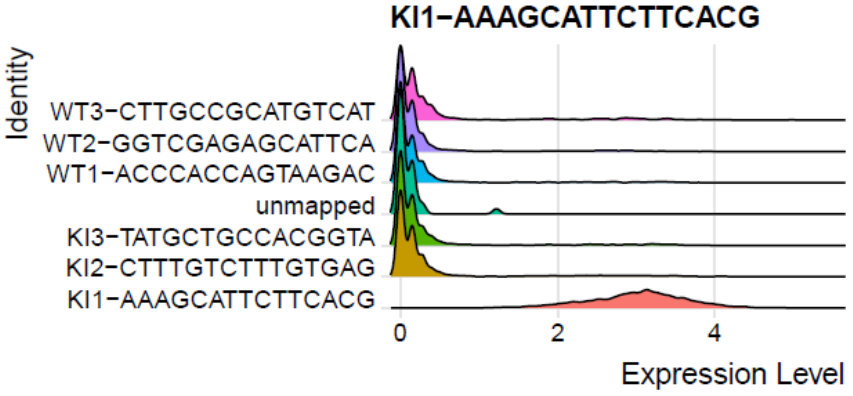
2078 cells 218 cells 12051 cells

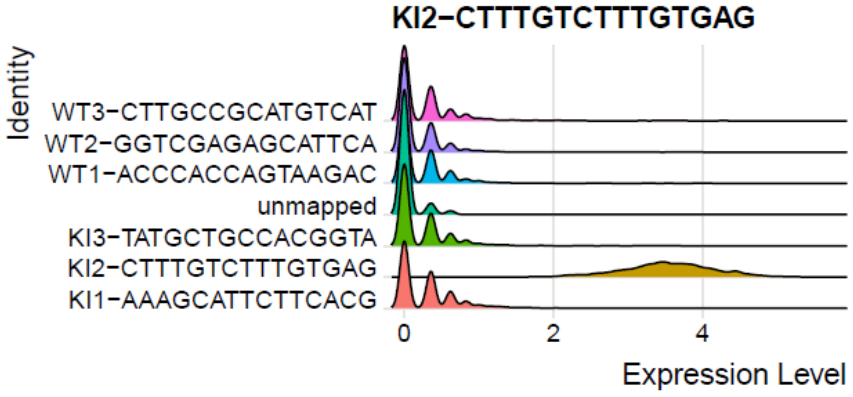
Ridge plots indicated the enrichment for selected HTOs

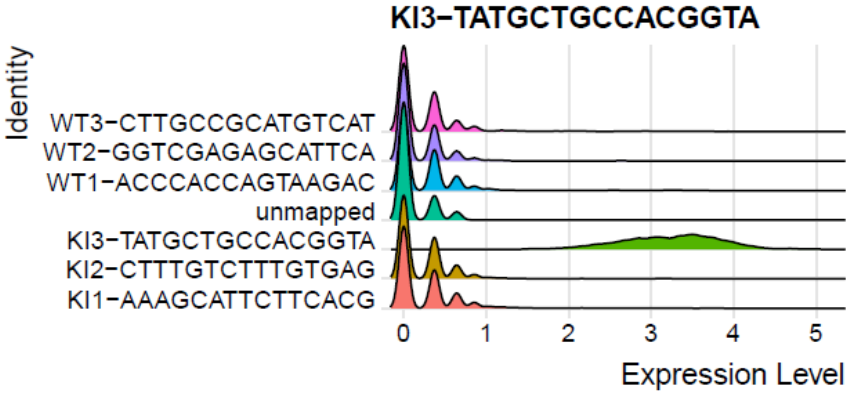




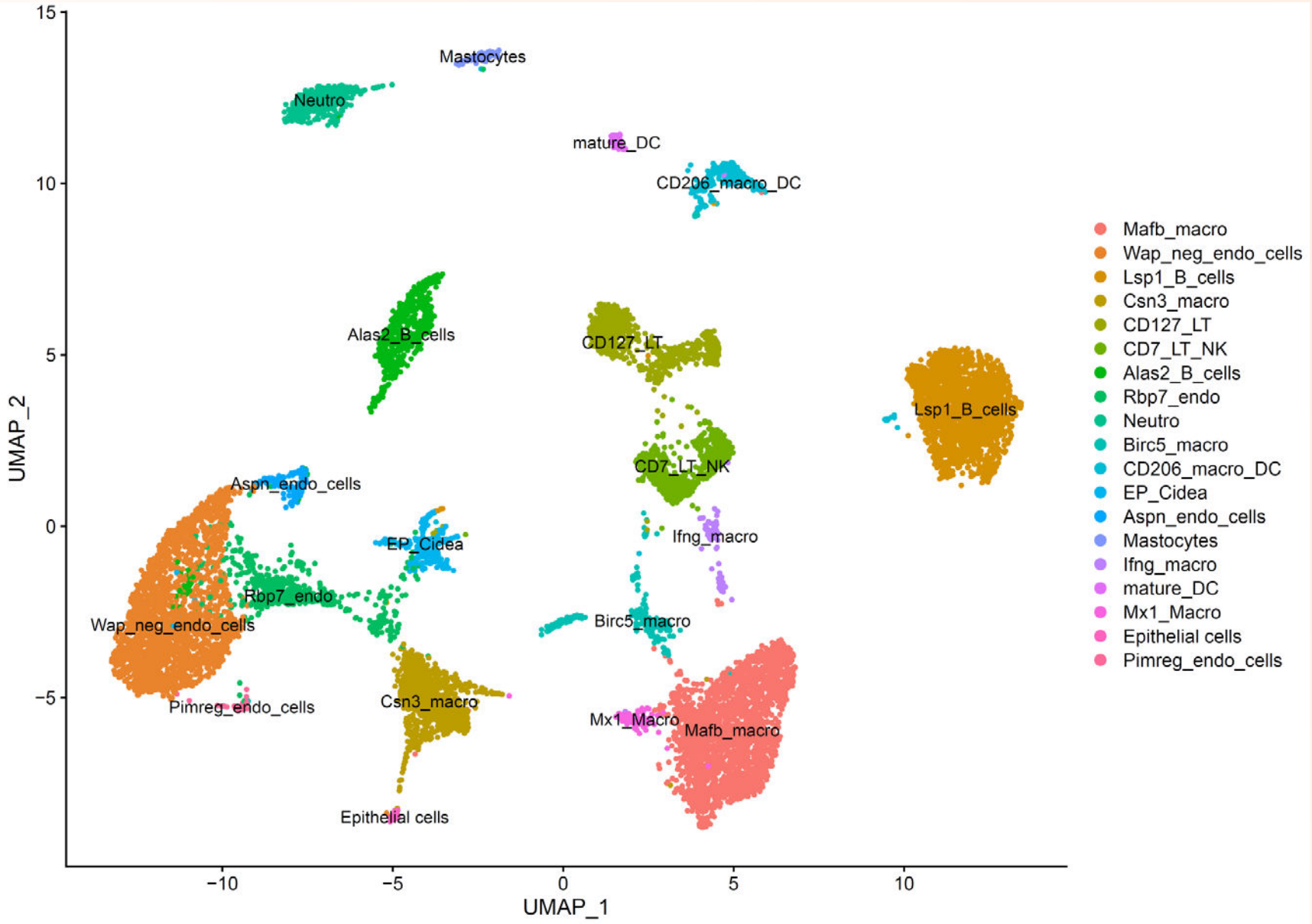




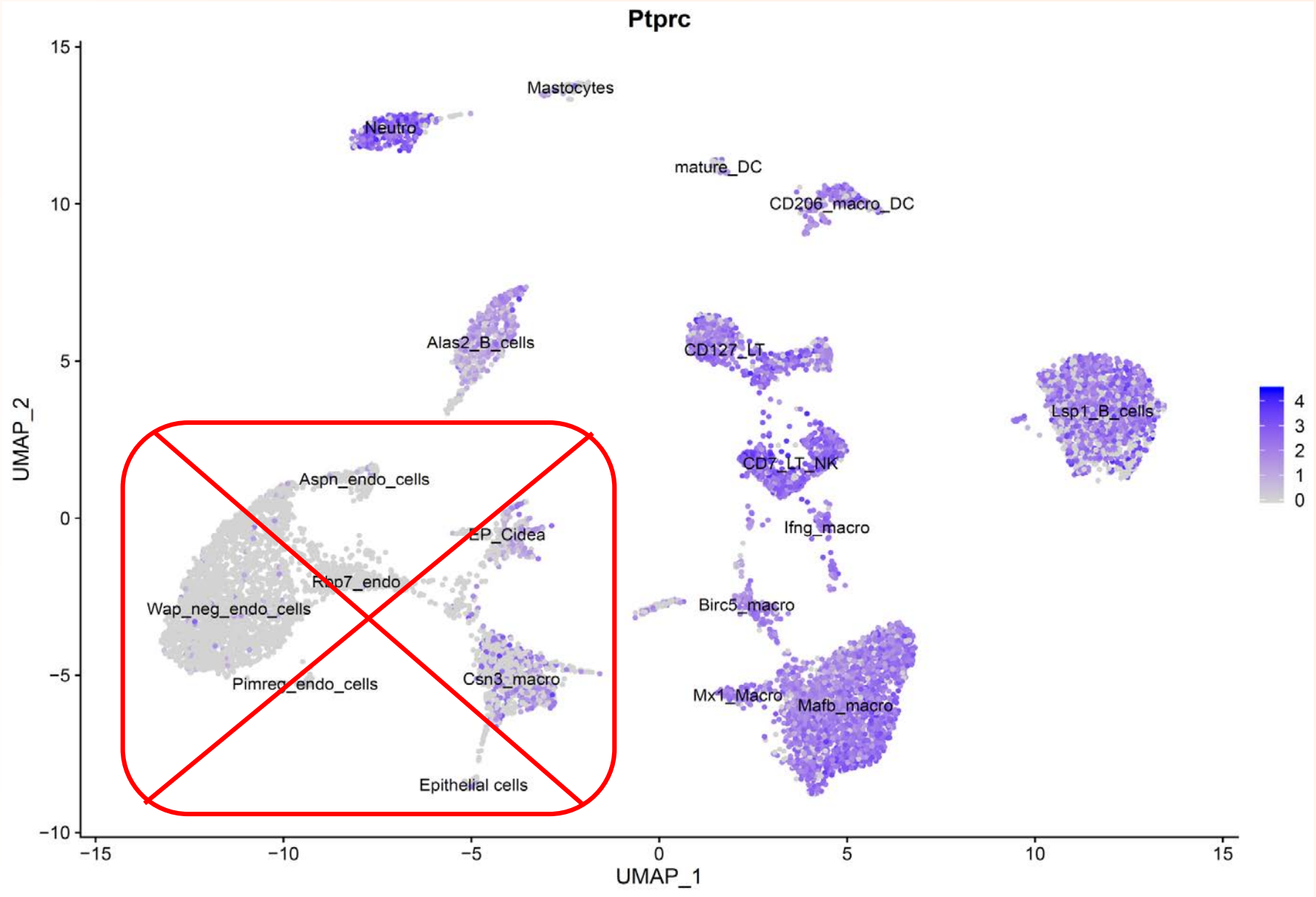




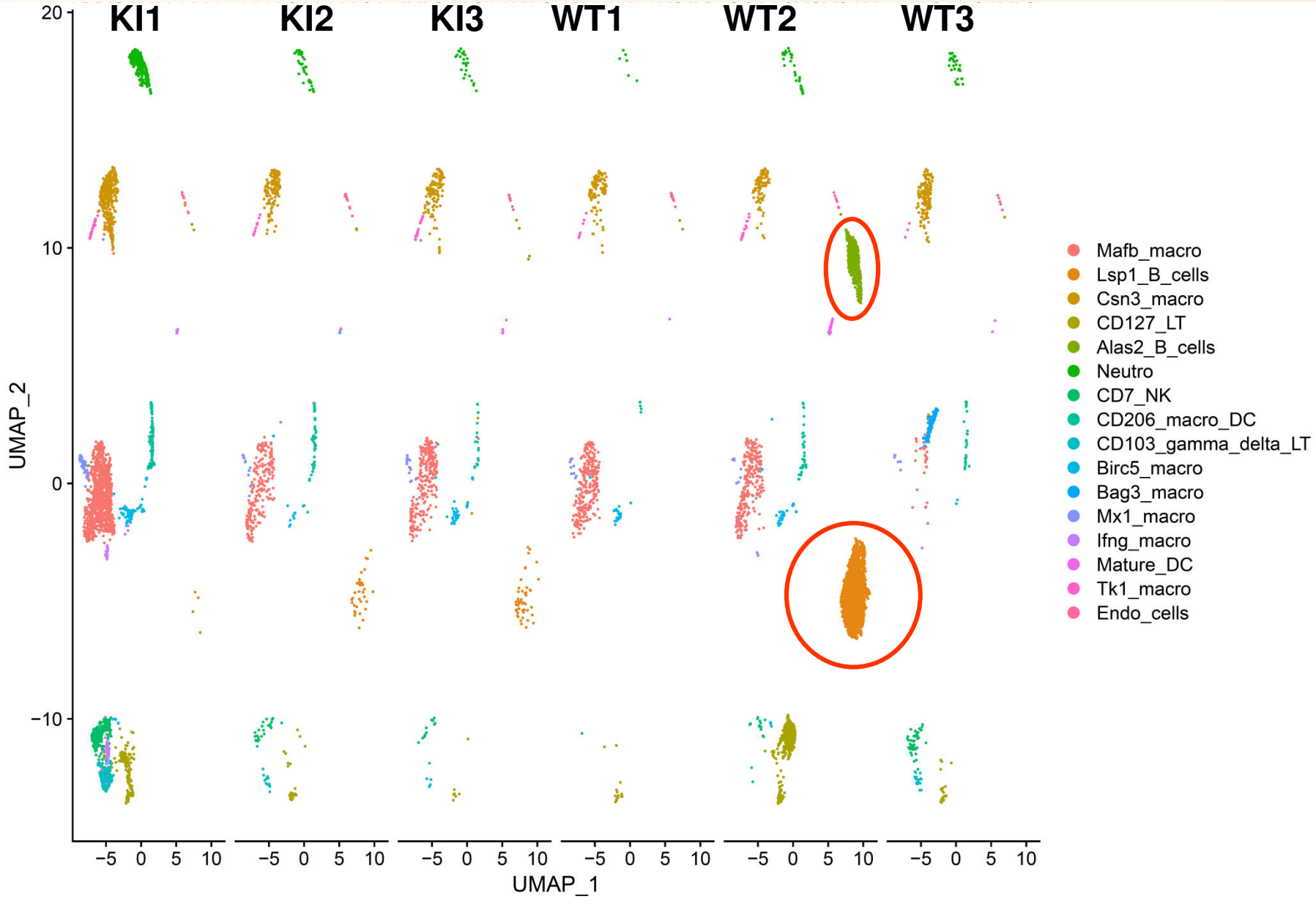
UMAP representation of the cell clusters (n=19) of all WT and KI mices



CD45 expression level in the cell clusters - All mices

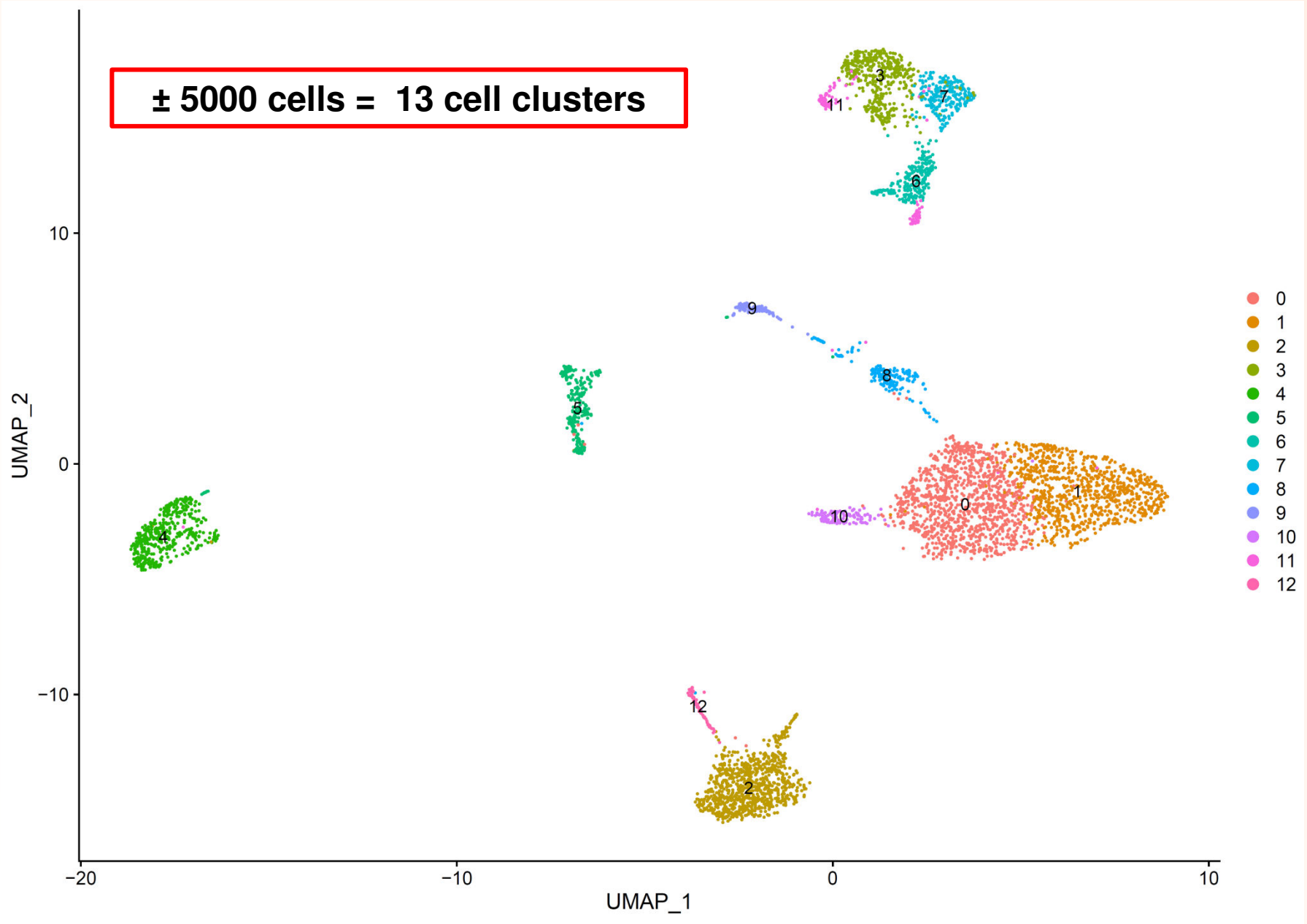


Heterogeneity of CD45 positive cells between mices

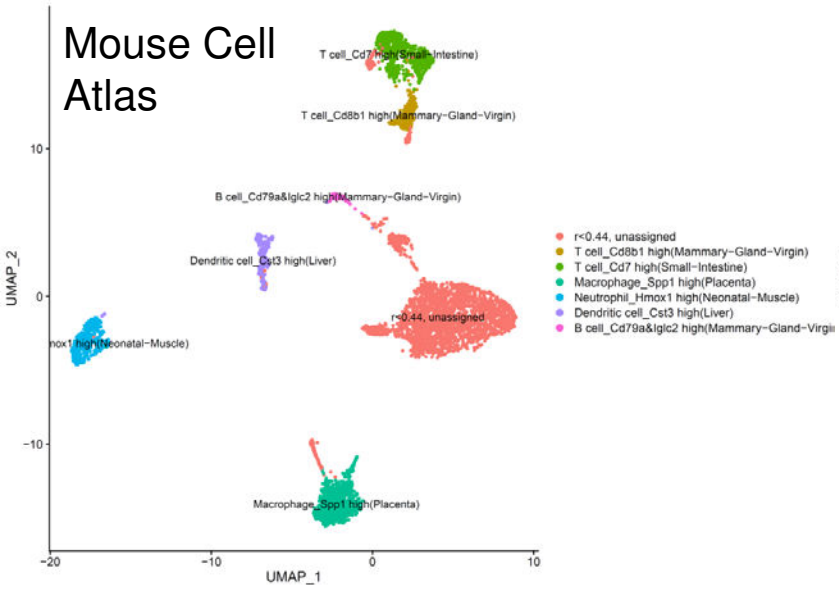


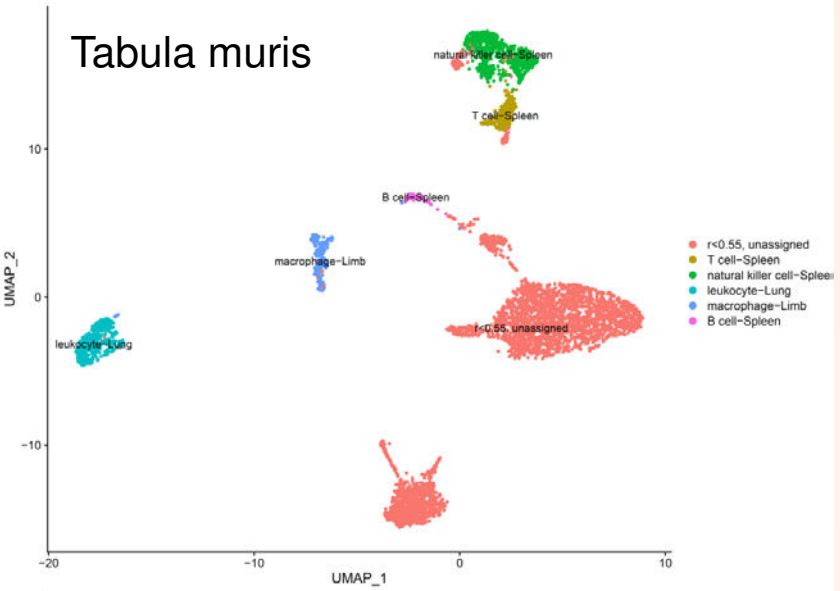
2913 cells | 560 cells | 597 cells | 449 cells | 3624 cells | 523 cells

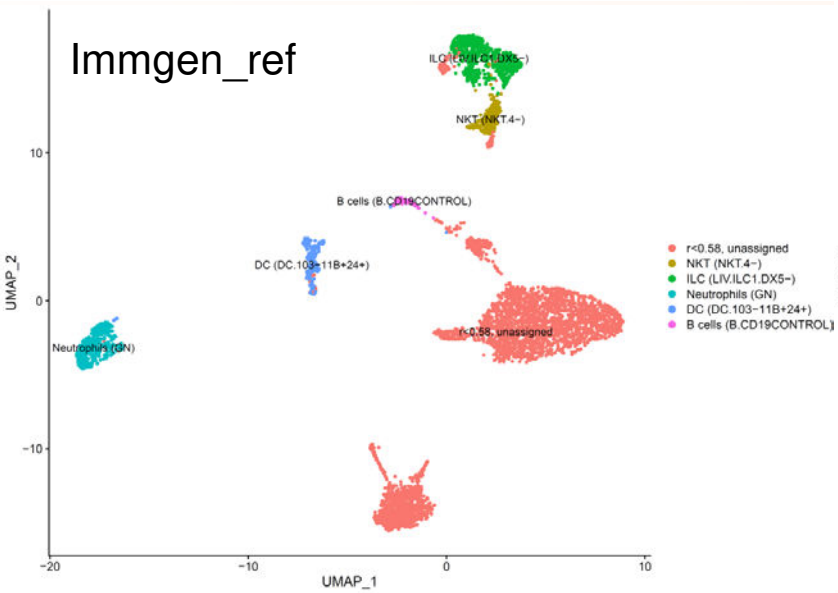
UMAP representation of CD45 positive cells – Without WT2 (LN)

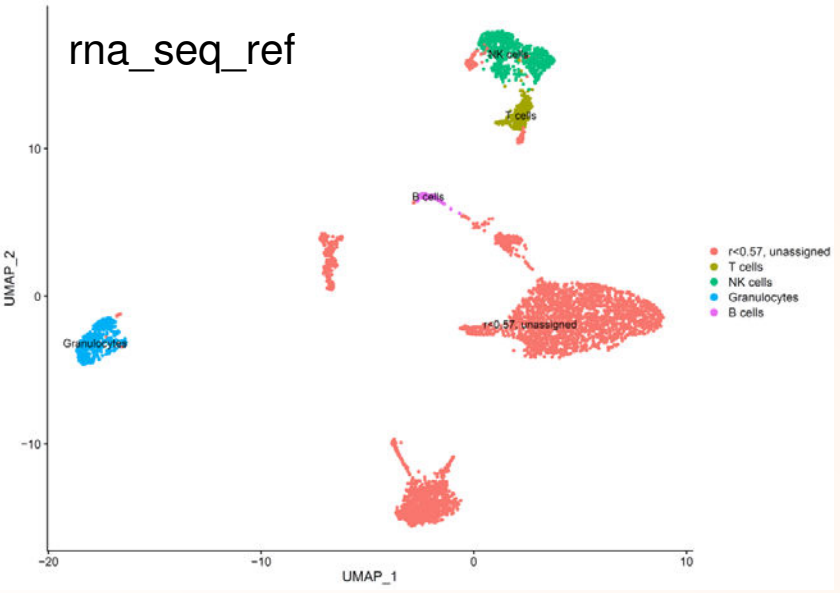


Clusters cell type automatic attribution: clustifyrdata



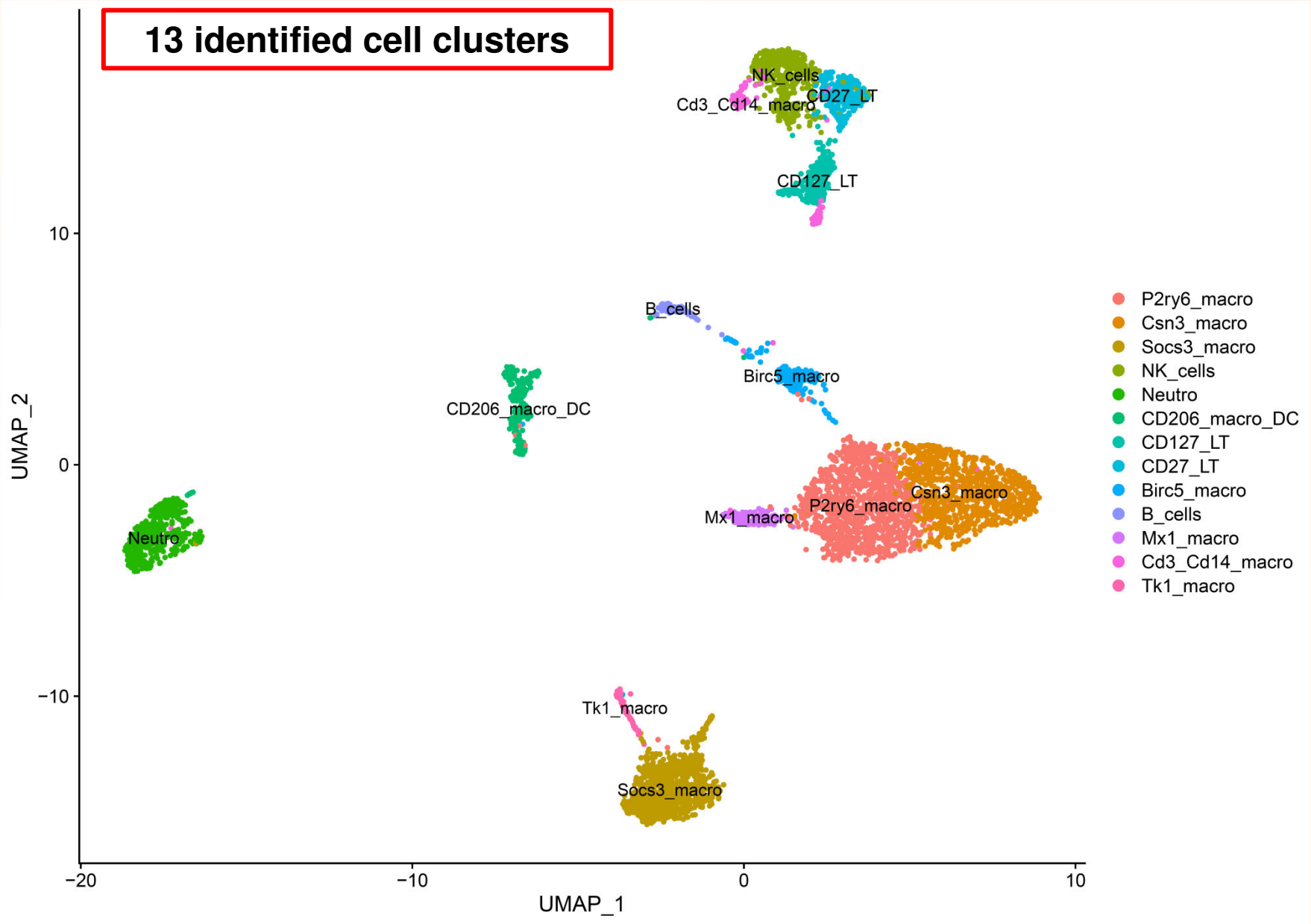




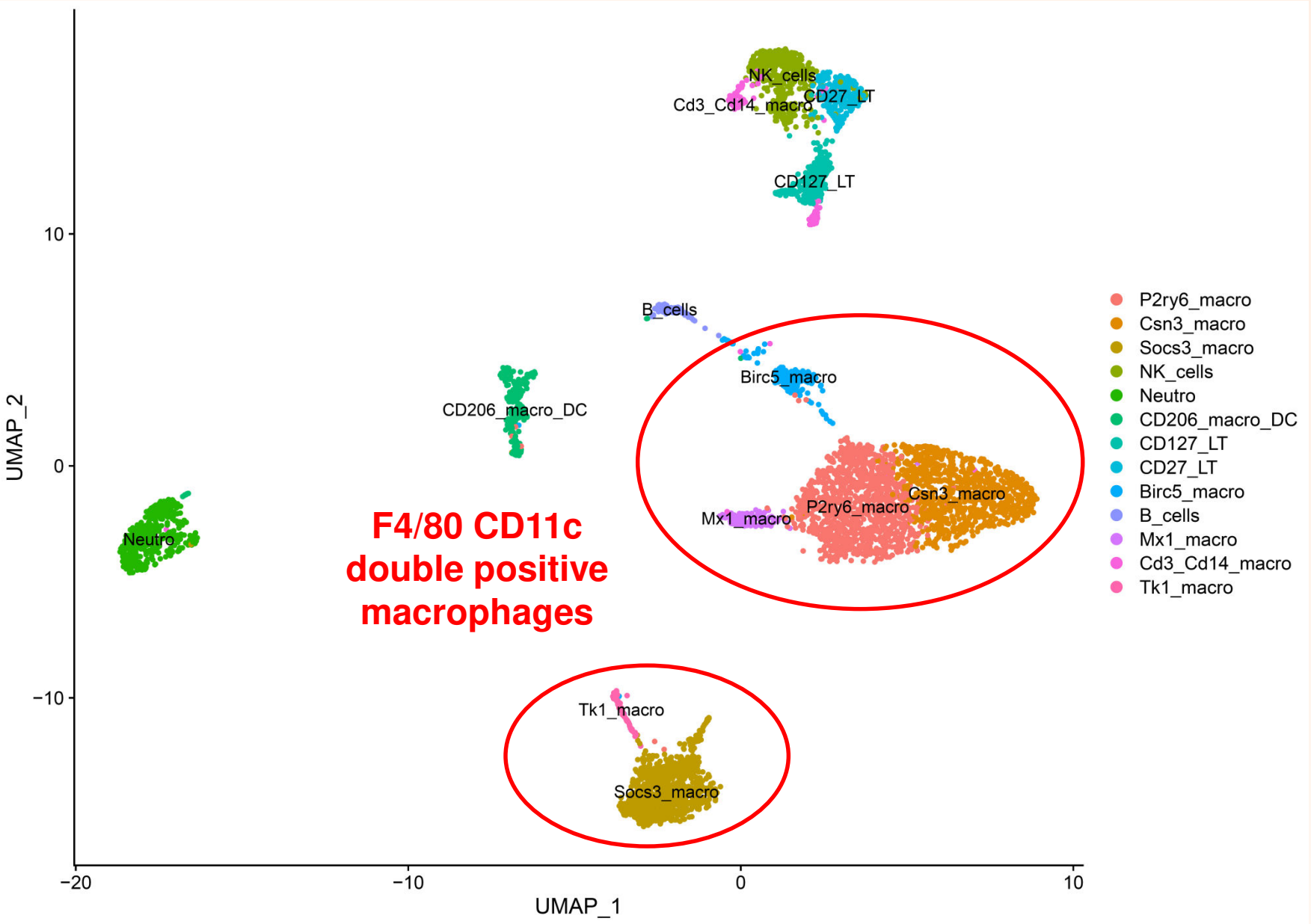


https://github.com/rnabioco/clustifyrdata

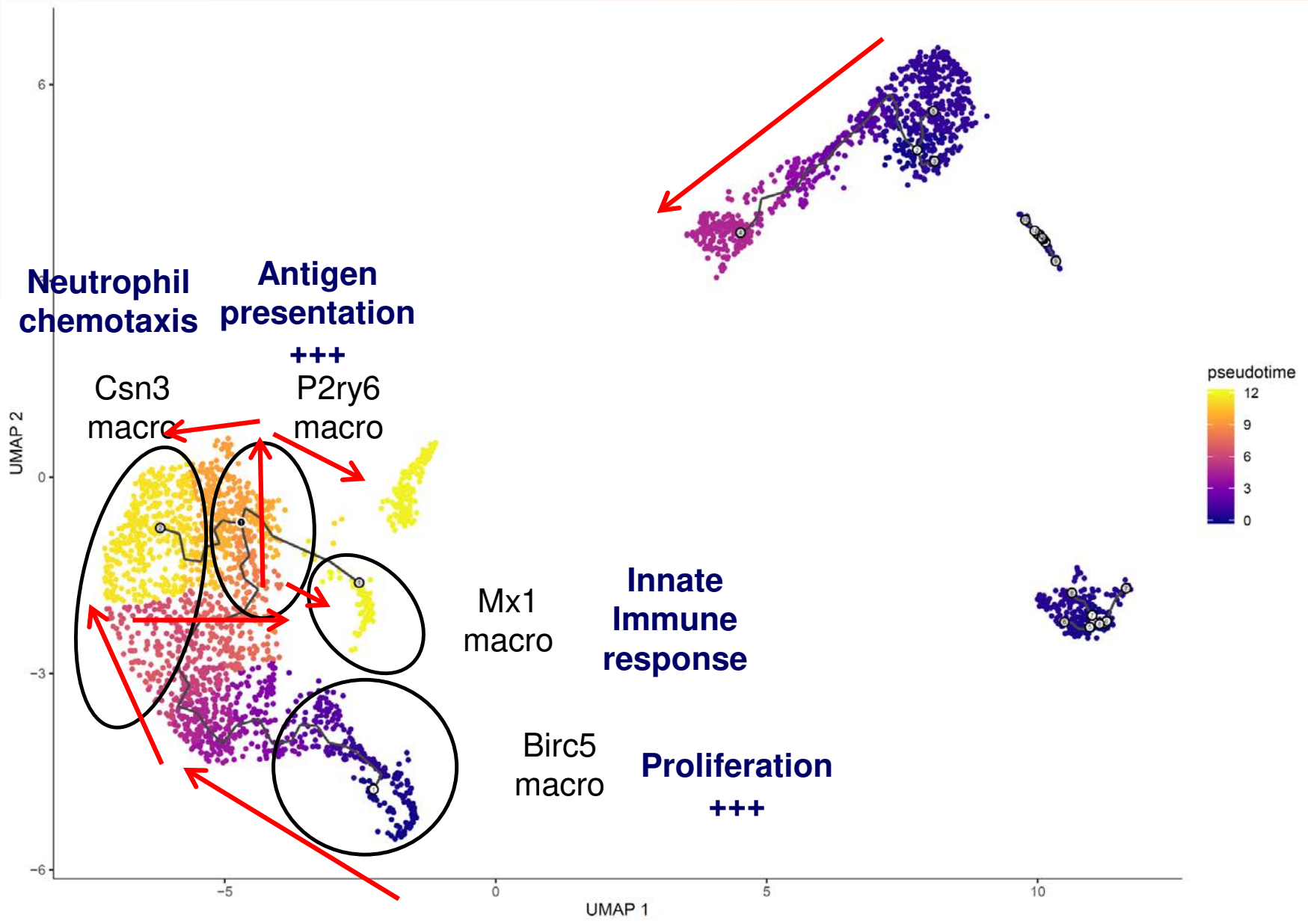
UMAP representation of CD45 positive cells – Without WT2 (LN)



Specific macrophages supopulations in lactating mammary gland?

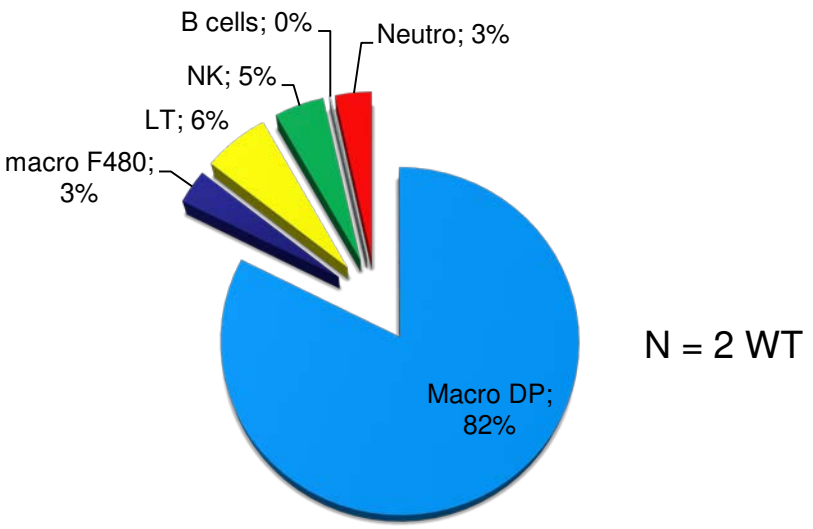


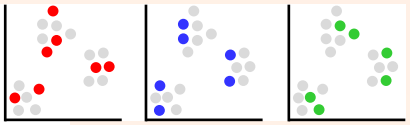
Monocle analysis on F4/80 CD11c macrophages supopulations



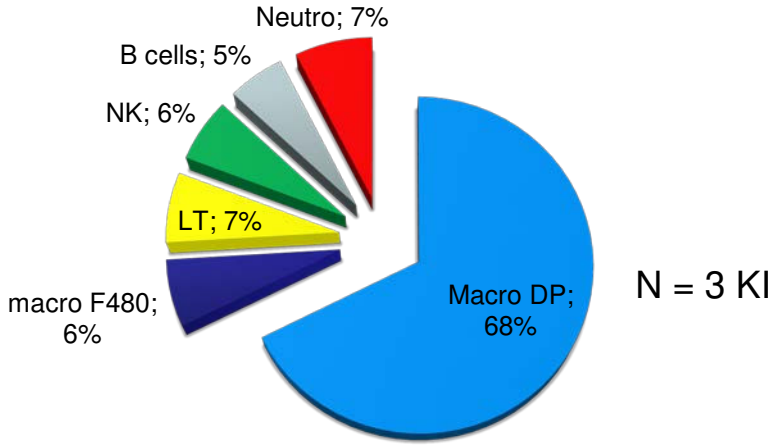
WT vs KI immune cell composition difference? – scRNA-Seq and HTO

WT





KI

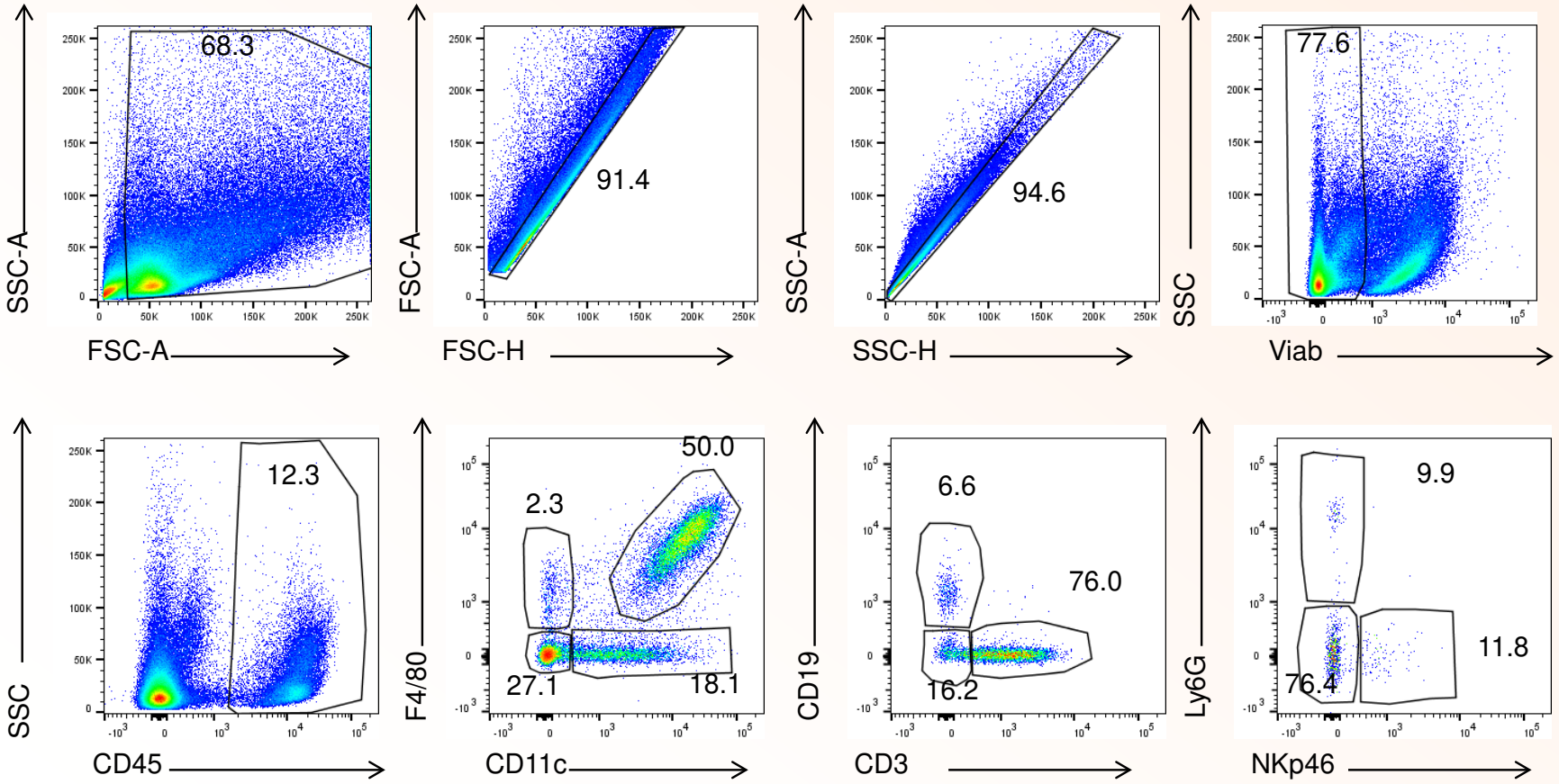


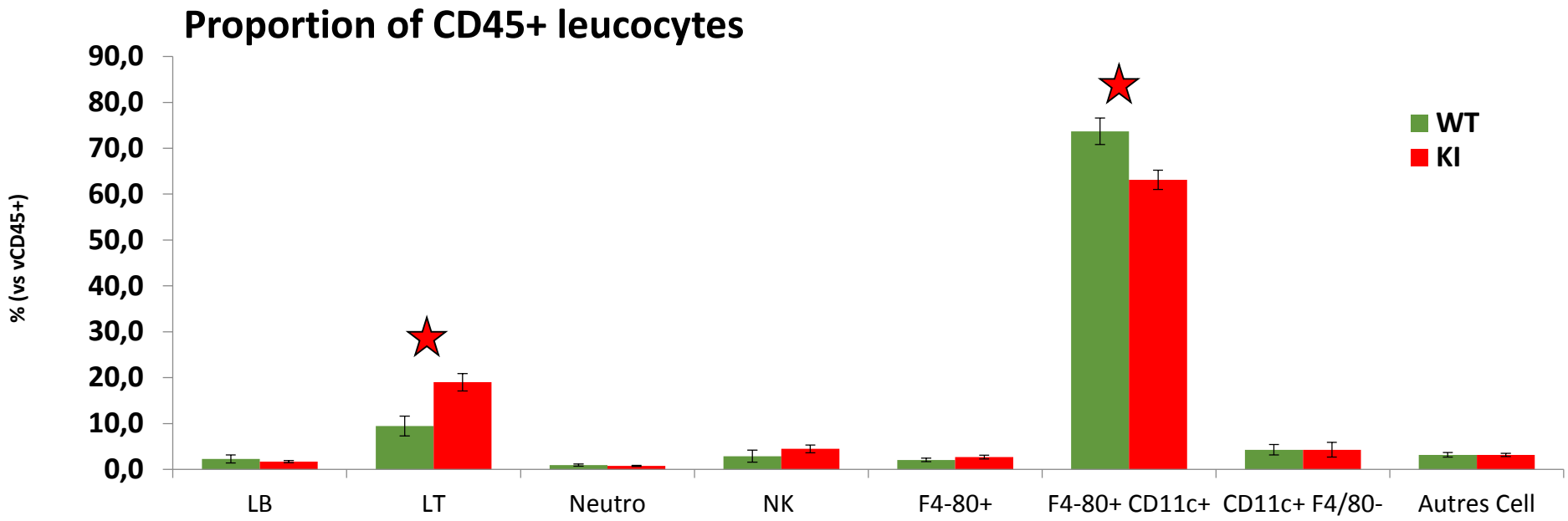
- **Macrophages and neutrophils altered in KI phenotype?**

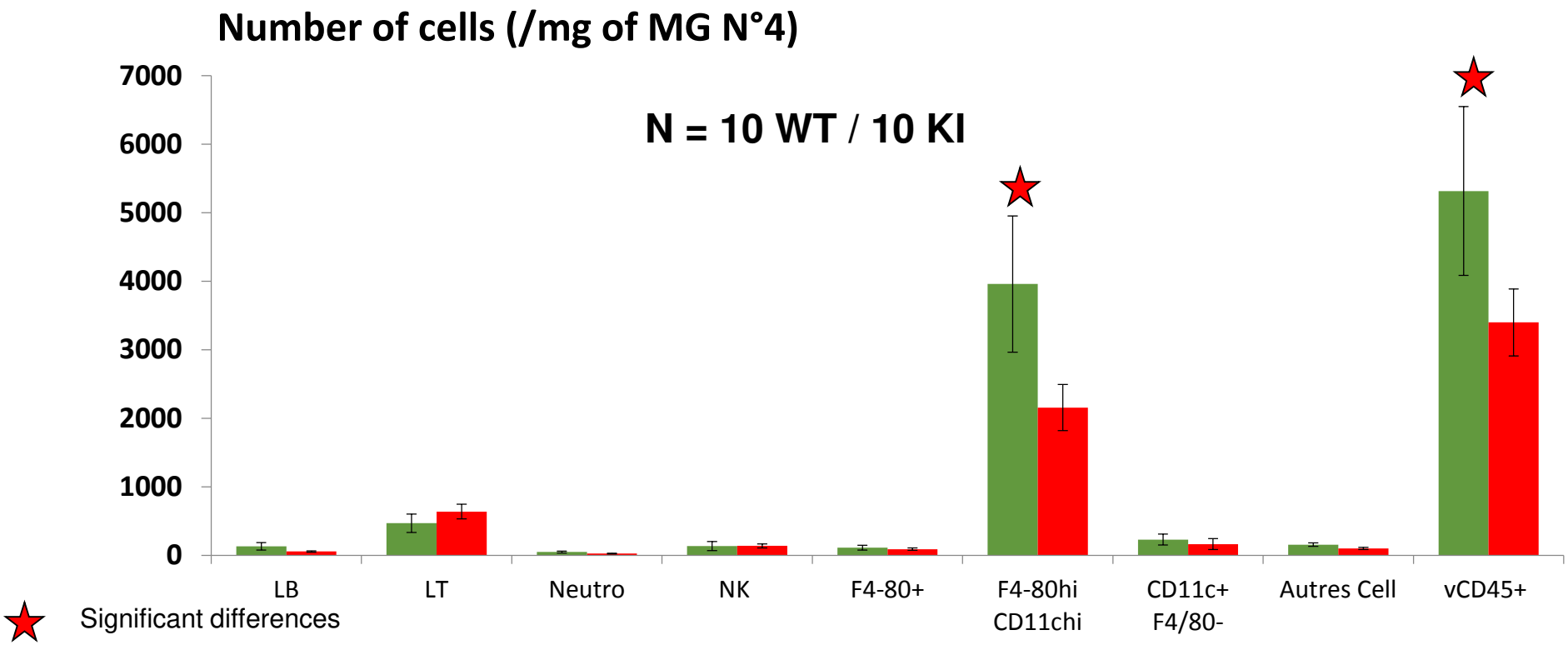
4) Immunophenotyping of SOCS2^{R96C} mice by flow cytometry

Phenotying analysis strategy by flow cytometry (Miltenyibiotec MACSQ10)

Lactating MG (d7) of females WT (n = 10) **vs KI** (n = 10)



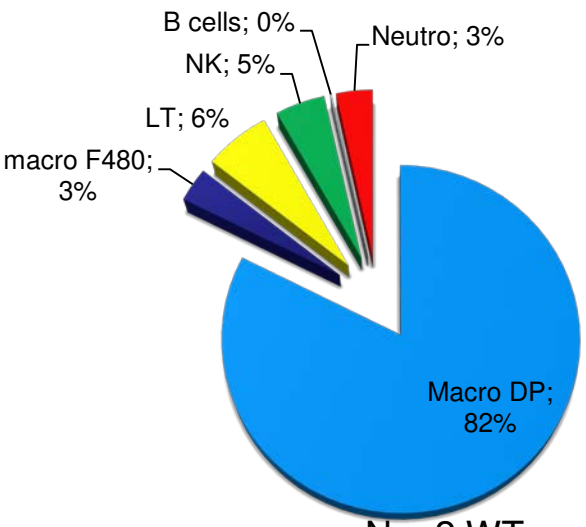

Proportion of CD45+ leucocytes
% (vs vCD45+)
90,0
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
WT
KI
LB
LT
Neutro
NK
F4-80+
F4-80+ CD11c+
CD11c+ F4/80-
Autres Cell


Number of cells (/mg of MG N°4)
N = 10 WT / 10 KI
7000
6000
5000
4000
3000
2000
1000
0
LB
LT
Neutro
NK
F4-80+
F4-80hi CD11chi
CD11c+ F4/80-
Autres Cell
vCD45+
Significant differences

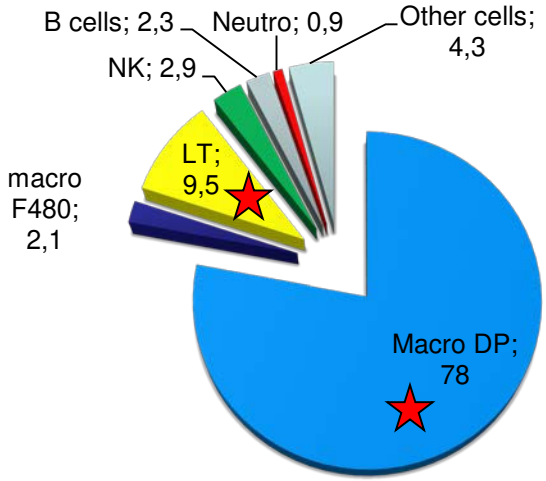
scRNA-Seq

Flow cytometry

WT

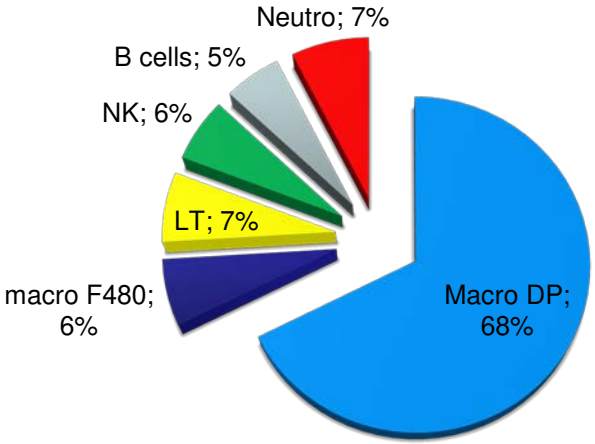


N = 2 WT

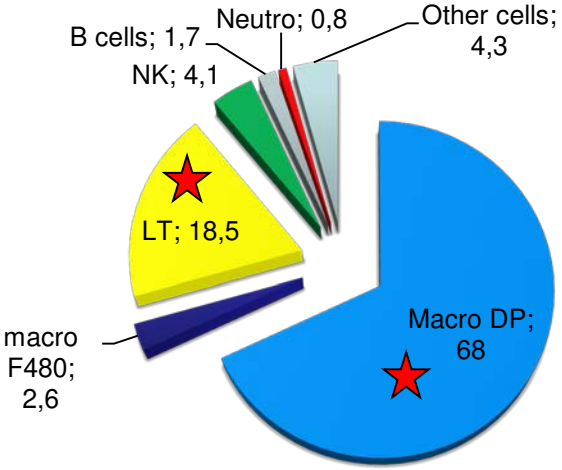


N = 10 WT

KI



N = 3 KI

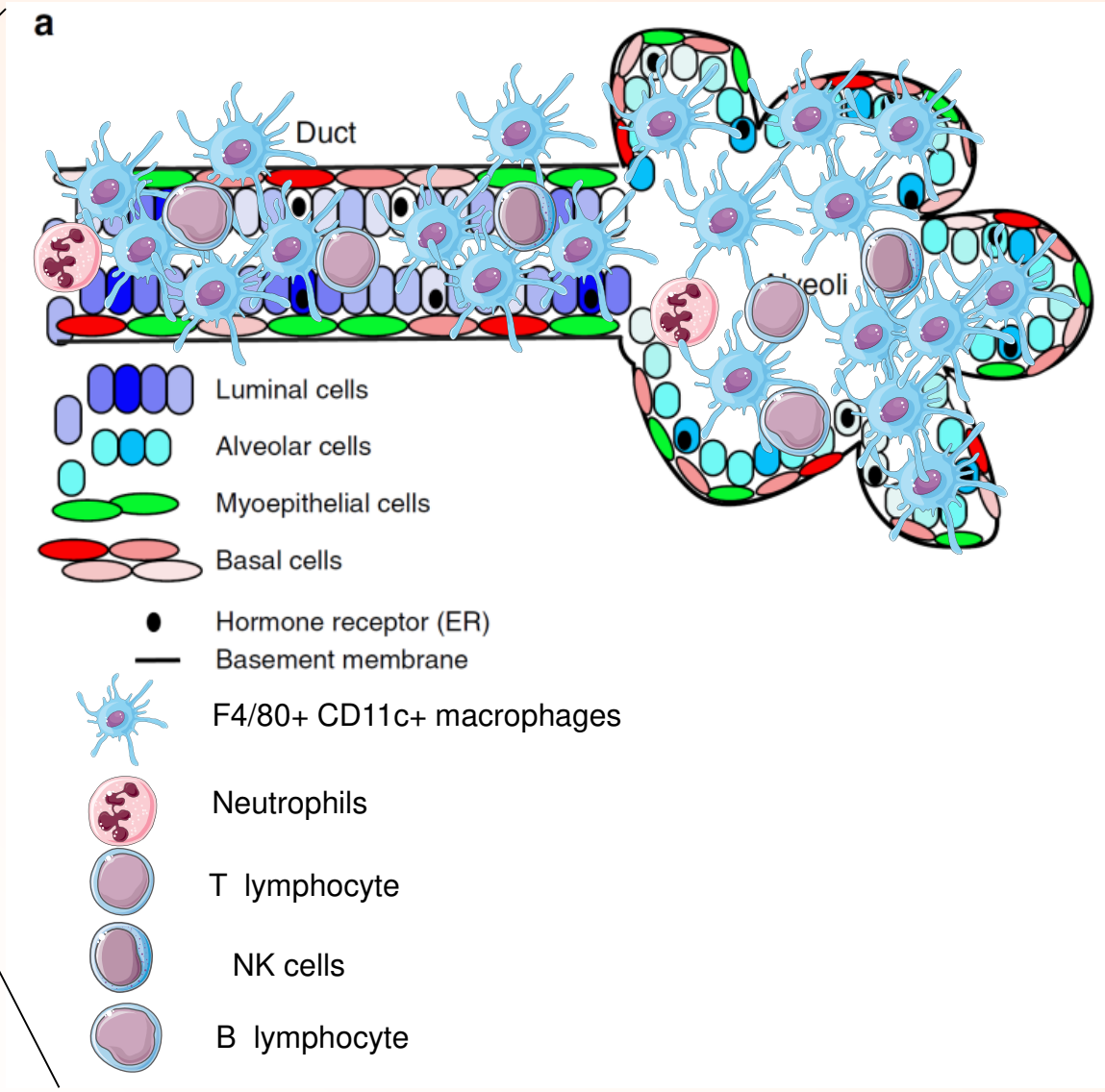


N = 10 KI

★ Significant differences

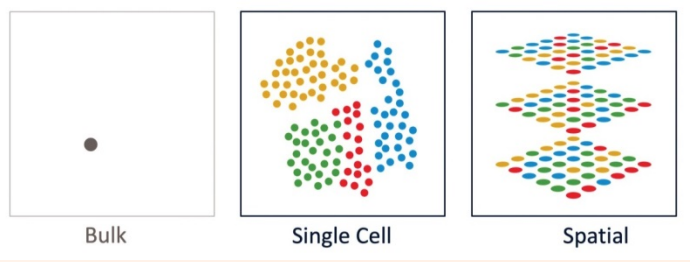
Conclusions

Lactating mammary gland



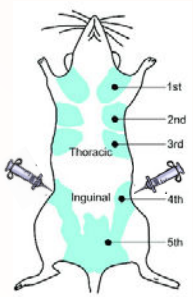
Cristea S, Polyak K. Dissecting the mammary gland one cell at a time. Nat Commun. 2018 Jun 26;9(1):2473.

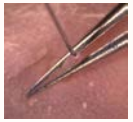
Prospects on REIDSOCS project



Identification of SOCS-2 function during Staphylococcus aureus infection in mice lactating mammary gland

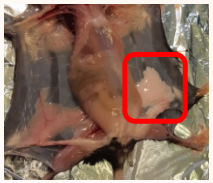
> **Lactating females** (7-10 days)





Intramammary injection of *Staphylococcus aureus*
(10^3 CFU/20 µL)

→

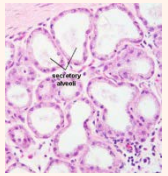


Lactating mammary gland dissection

→

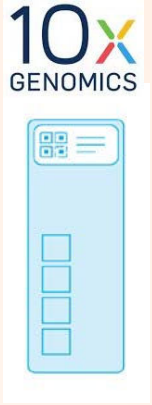
Inclusion in OCT

→



Tissue section of lactating mammary gland

→



Spatial transcriptomic

➢ Determine the transcriptome of both immune and non immune lactating mammary gland cells during bacterial infection (Staphylococcus aureus) with spatial information

Collaboration with Spatial Transcriptomics Pilot Facility (Annelie Mollbrink), Sweden

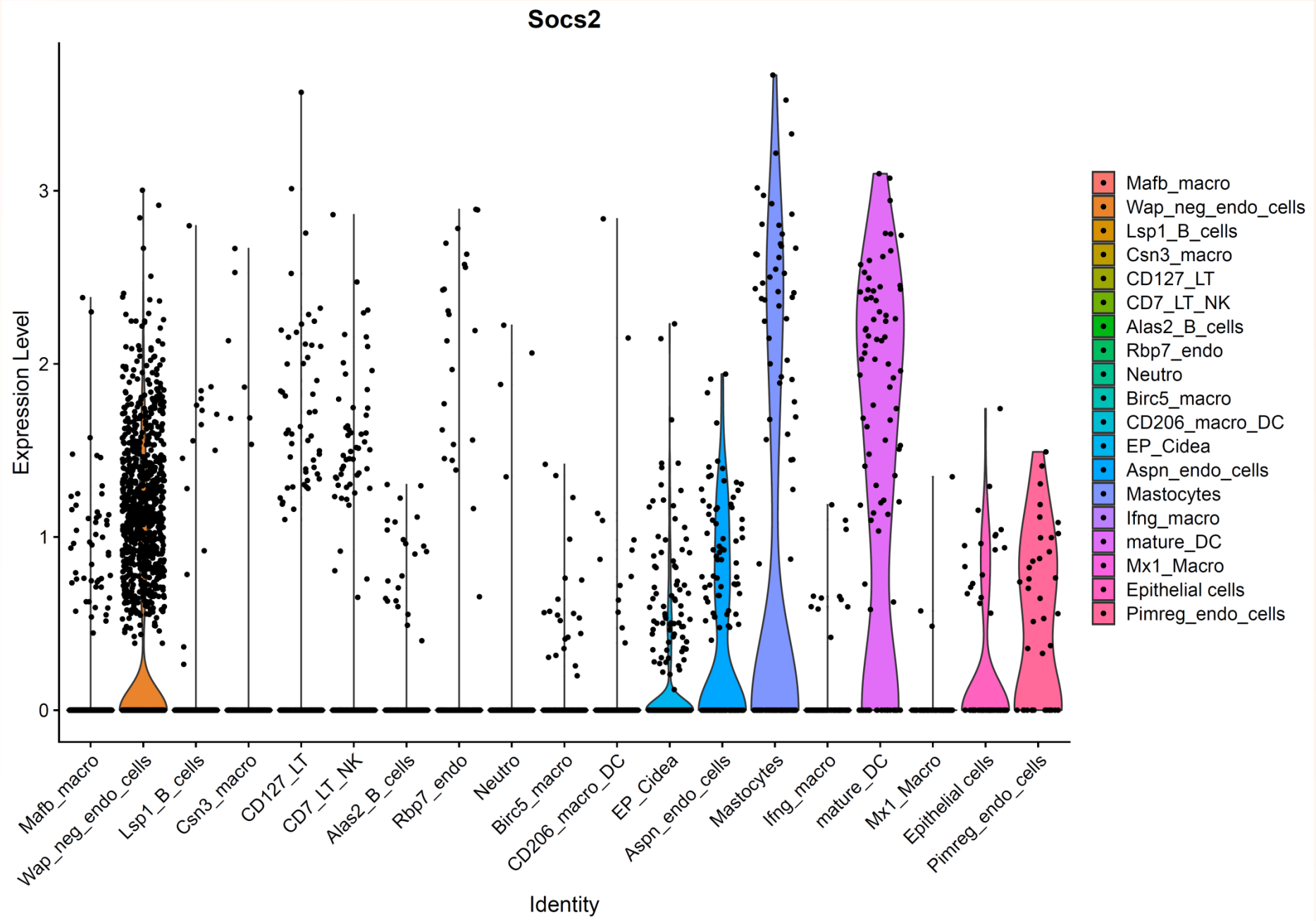
THANK YOU FOR YOUR ATTENTION

Team : IALTA

Team leader : Pr Gilles Foucras

UMR INRA ENVT 1225 – IHAP

- Gilles FOUCRAS, PR
- Guillaume TABOURET, CR
- Laurence GUZYLACK-PIRIOU, CR
- Chervin HASSEL, IR post doc
- Nathan CEBRON, Doctorant
- Blandine GAUSSERES, IE
- Julie COURNET, TR
- Christian TASCA, TR


Socs2
Expression Level
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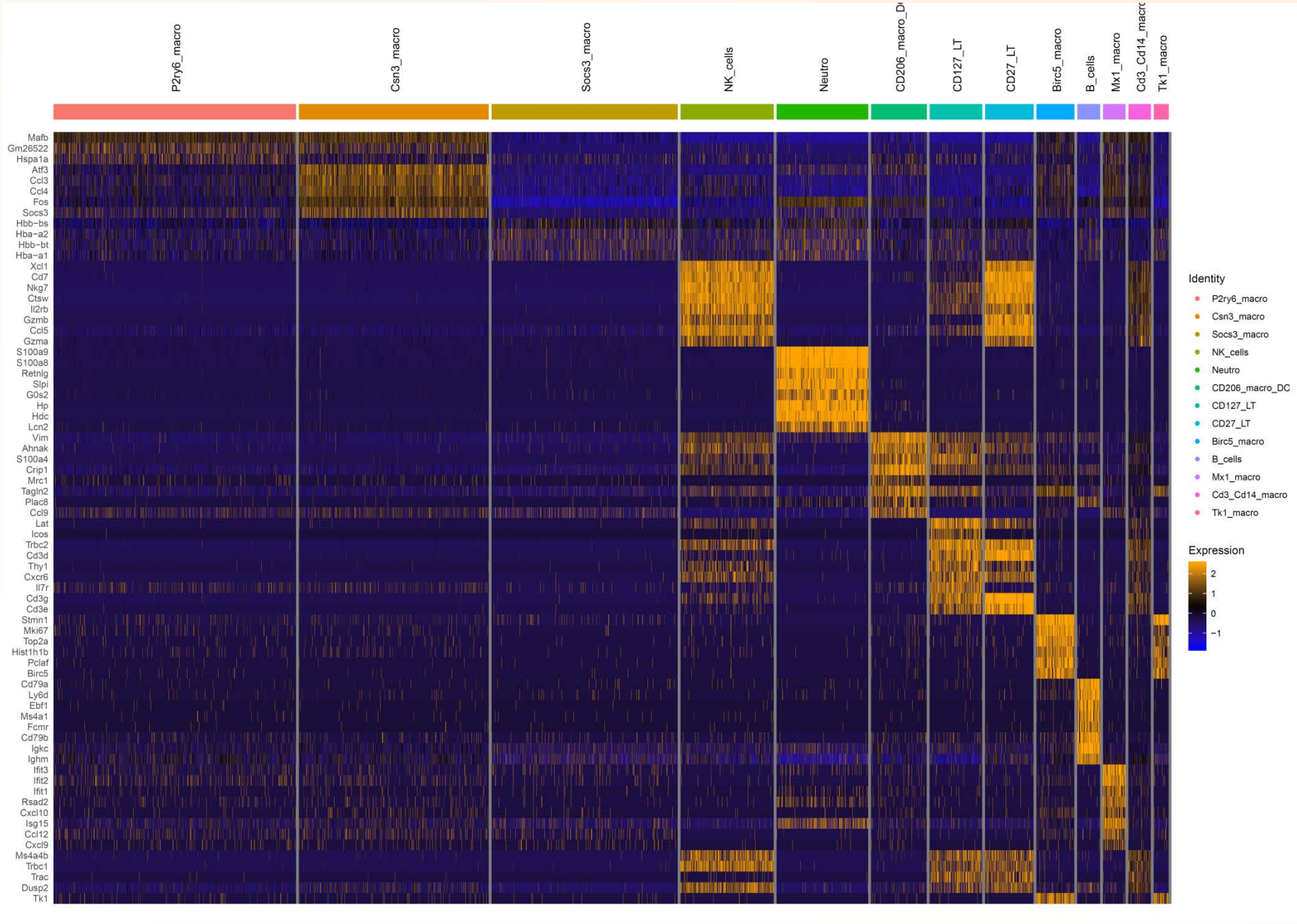
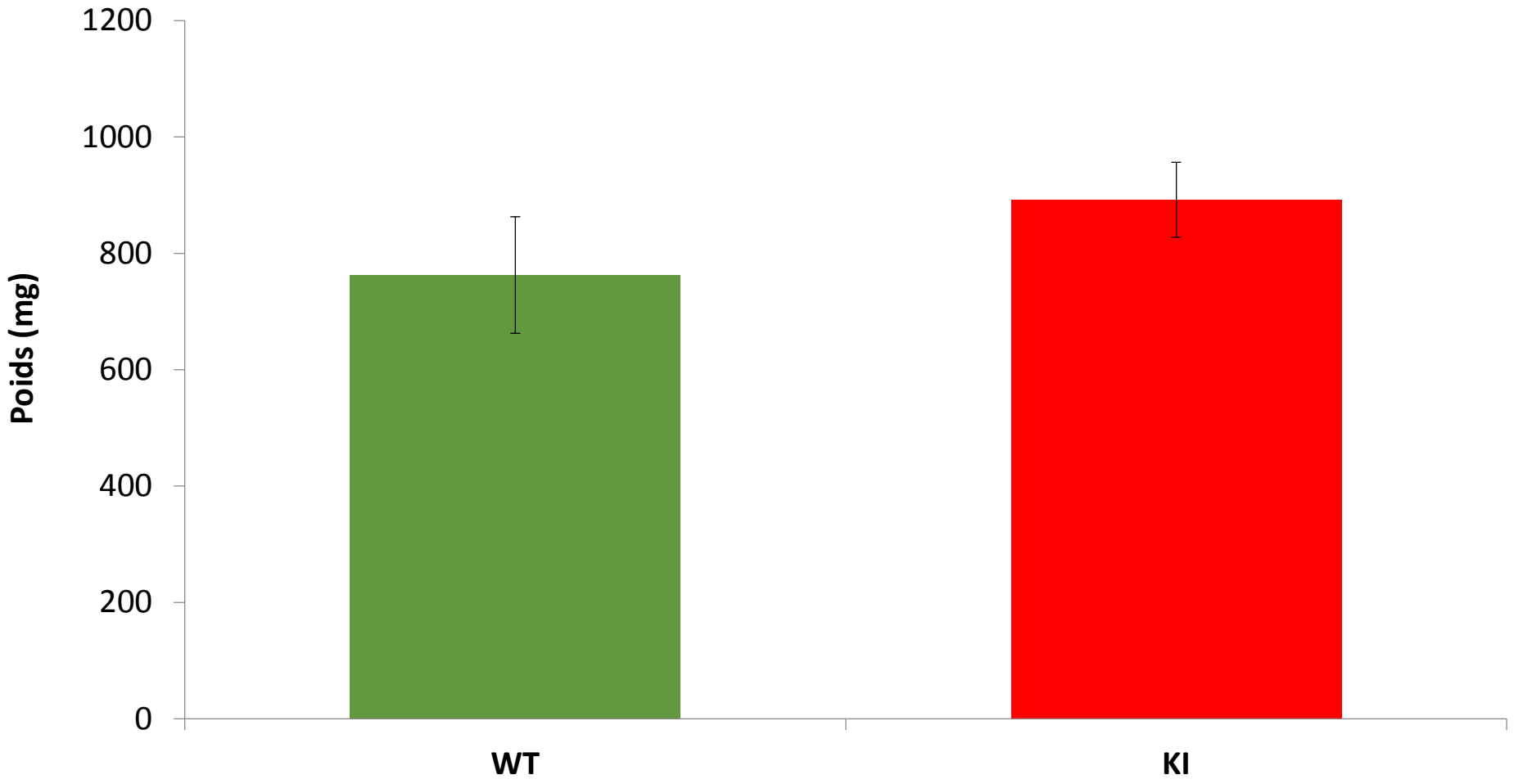
1
2
3
Identity
Mafb_macro
Wap_neg_endo_cells
Lsp1_B_cells
Csn3_macro
CD127_LT
CD7_LT_NK
Alas2_B_cells
Rbp7_endo
Neutro
Birc5_macro
CD206_macro_DC
EP_Cidea
Aspn_endo_cells
Mastocytes
Ifng_macro
mature_DC
Mx1_Macro
Epithelial cells
Pimreg_endo_cells

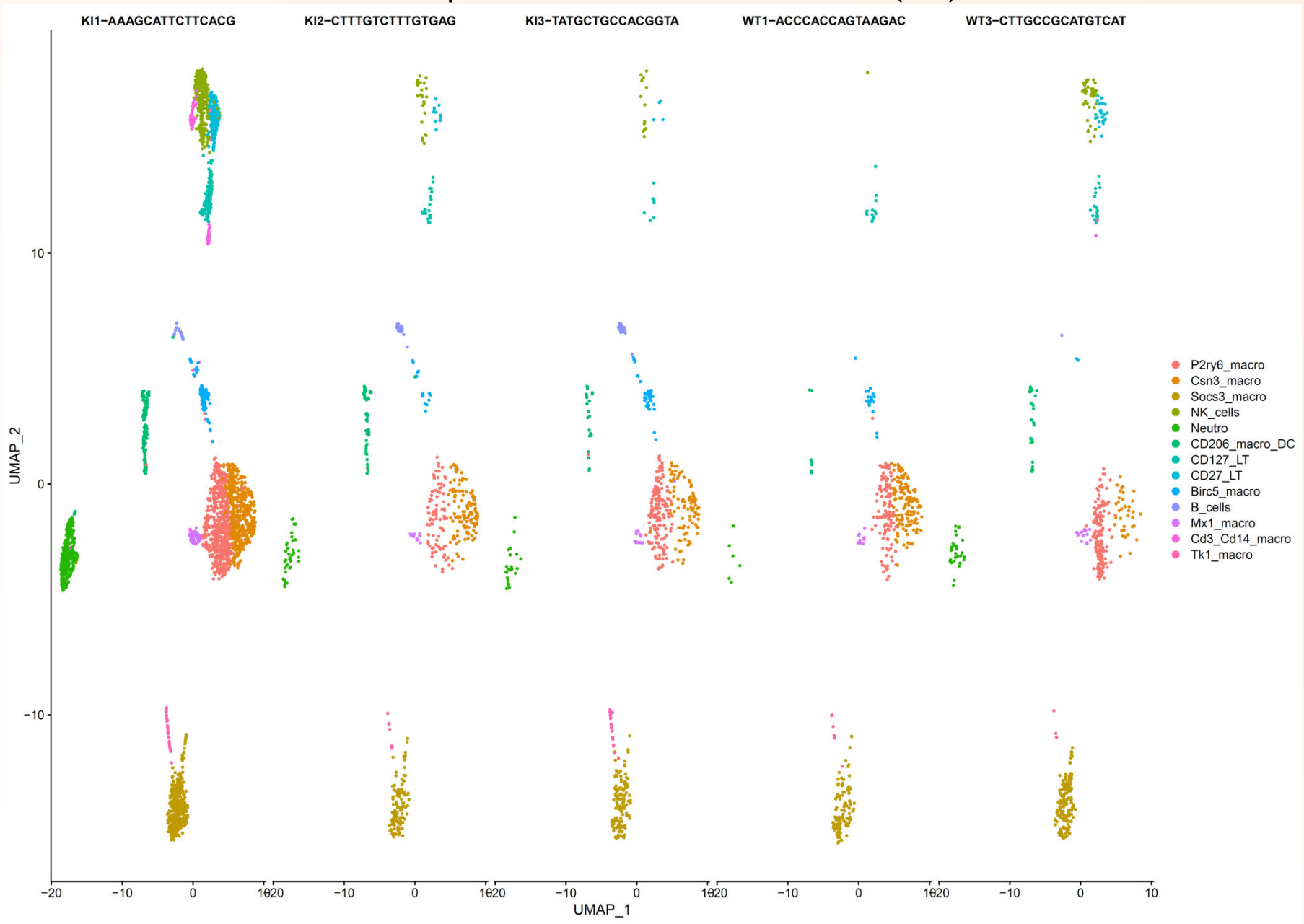

P2ry6_macro
Csn3_macro
Socs3_macro
NK_cells
Neutro
CD206_macro_D
CD127_LT
CD27_LT
Birc5_macro
B_cells
Mx1_macro
Cd3_Cd14_macro
Tk1_macro
Mafb
Gm26522
Hspa1a
Atf3
Ccl3
Ccl4
Fos
Socs3
Hbb-bs
Hba-a2
Hbb-bt
Hba-a1
Xcl1
Cd7
Nkg7
Ctsw
Il2rb
Gzmb
Ccl5
Gzma
S100a9
S100a8
Retnlg
Slpi
G0s2
Hp
Hdc
Lcn2
Vim
Ahnak
S100a4
Cnip1
Mrc1
Tagln2
Plac8
Ccl9
Lat
Icos
Trbc2
Cd3d
Thy1
Cxcr6
Il7r
Cd3g
Cd3e
Stmn1
Mki67
Top2a
Hist1h1b
Pclaf
Birc5
Cd79a
Ly6d
Ebf1
Ms4a1
Fcmr
Cd79b
Igkc
Ighm
Ifit3
Ifit2
Ifit1
Rsad2
Cxcl10
Isg15
Ccl12
Cxcl9
Ms4a4b
Trbc1
Trac
Dusp2
Tk1
Identity
P2ry6_macro
Csn3_macro
Socs3_macro
NK_cells
Neutro
CD206_macro_DC
CD127_LT
CD27_LT
Birc5_macro
B_cells
Mx1_macro
Cd3_Cd14_macro
Tk1_macro
Expression
2
1
0
−1

Poids des 2 glandes mammaires N°4



N = 5 WT / 8 KI

CD45 positive cells – Without WT2 (LN)



2913 cells | 560 cells | 597 cells | 449 cells | 523 cells

Description	P-value	FDR q-value	Cluster
immune response	2.87E-4	1	P2ry6_macro
antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	5.09E-4	1	P2ry6_macro
antigen processing and presentation of peptide antigen via MHC class II	5.09E-4	7.79E-1	P2ry6_macro
antigen processing and presentation of peptide antigen	6.36E-4	7.3E-1	P2ry6_macro
antigen processing and presentation	6.36E-4	5.84E-1	P2ry6_macro
cytokine-mediated signaling pathway	6.81E-5	2.26E-1	Csn3_macro
signal transduction	1.82E-4	3.02E-1	Csn3_macro
granulocyte chemotaxis	2.3E-4	2.55E-1	Csn3_macro
neutrophil chemotaxis	2.3E-4	1.91E-1	Csn3_macro
neutrophil migration	2.3E-4	1.53E-1	Csn3_macro
myeloid leukocyte migration	2.3E-4	1.27E-1	Csn3_macro
cellular amide metabolic process	4.68E-6	8,00E-03	Socs3_macro
translation	1.14E-5	9.77E-3	Socs3_macro
amide biosynthetic process	1.14E-5	6.51E-3	Socs3_macro
peptide metabolic process	1.14E-5	4.88E-3	Socs3_macro
peptide biosynthetic process	1.14E-5	3.91E-3	Socs3_macro
negative regulation of developmental process	1.66E-4	6.5E-1	CD206_macro
cell differentiation	3.14E-4	6.15E-1	CD206_macro
positive regulation of chemotaxis	7.01E-4	9.15E-1	CD206_macro
regulation of anatomical structure morphogenesis	8.07E-4	7.9E-1	CD206_macro
negative regulation of cell proliferation	9.68E-4	7.58E-1	CD206_macro
cell cycle	2.83E-20	8.48E-17	Birc5_macro
cell cycle process	5.36E-20	8.02E-17	Birc5_macro
cell division	3.25E-16	3.24E-13	Birc5_macro
mitotic cell cycle process	1.54E-14	1.15E-11	Birc5_macro
regulation of cell cycle	2.52E-13	1.51E-10	Birc5_macro
regulation of cell cycle process	5.52E-10	2.76E-7	Birc5_macro
defense response	9.87E-11	6.52E-8	Mx1_macro
response to other organism	5.48E-10	1.81E-7	Mx1_macro
defense response to virus	1.16E-9	3.29E-7	Mx1_macro
response to virus	5.79E-9	1.43E-6	Mx1_macro
defense response to other organism	1.57E-8	3.45E-6	Mx1_macro
innate immune response	2.33E-8	4.62E-6	Mx1_macro
immune effector process	3.23E-8	5.81E-6	Mx1_macro
immune response	3.3E-8	5.45E-6	Mx1_macro
immune system process	1.62E-7	2.47E-5	Mx1_macro
response to bacterium	7.38E-5	9.14E-3	Mx1_macro
cellular response to interferon-alpha	1.16E-4	1.35E-2	Mx1_macro
regulation of innate immune response	1.44E-4	1.59E-2	Mx1_macro

CD45 positive cells – Without WT2 (LN)

	WT1	WT3	KI1	KI2	KI3	WT	KI
P2ry6_macro	28%	31%	20%	17%	27%	**29%**	**21%**
Csn3_macro	32%	7%	17%	21%	17%	**20%**	**18%**
Socs3_macro	25%	30%	12%	21%	21%	**27%**	**18%**
NK_cells	0%	9%	12%	5%	2%	5%	6%
Neutro	1%	5%	11%	7%	4%	**3%**	**7%**
CD206_macro_DC	2%	5%	5%	10%	4%	3%	6%
CD127_LT	4%	5%	6%	4%	1%	4%	4%
CD27_LT	0%	4%	7%	2%	1%	2%	3%
Birc5_macro	5%	0%	3%	3%	7%	3%	4%
B_cells	0%	0%	0%	7%	9%	0%	5%
Mx1_macro	2%	3%	2%	2%	2%	2%	2%
Cd3_Cd14_macro	0%	0%	4%	0%	0%	0%	1%
Tk1_macro	1%	1%	1%	1%	4%	1%	2%

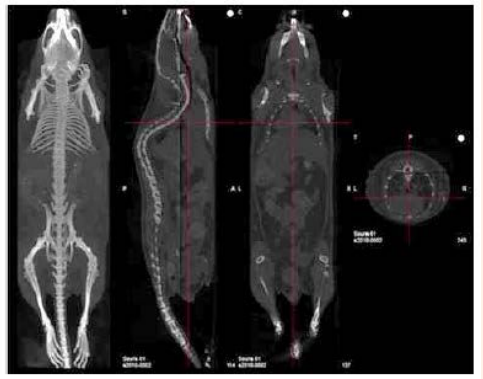
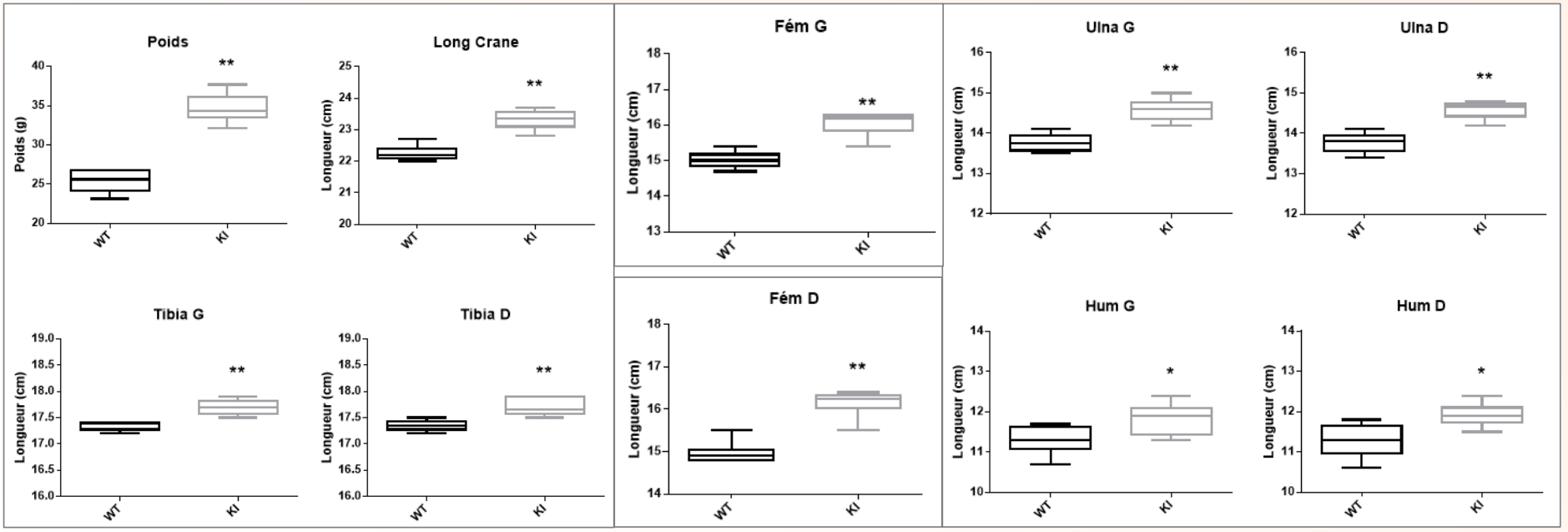
Immunophenotyping of SOCS2^{R96C} mice by flow cytometry

Phenotying analysis strategy by flow cytometry
(Miltenyi MACSQUANT10)

	Fluorochome	Target	Cells
V1	Pacific blue	Ly-6G	Neutrophils
V2	VioGreen	CD45	Immune cells
B1	FITC	Viobility Fixable Dye	Dead cells
B2	PE	Nkp46 (CD335)	NK cells
B3	PE-Vio615	CD19	LB
B4	PeCy7	CD11c	Myeloid cells (DC / macro)
R1	APC	F4/80	macrophages
R2	APC-Vio770	CD3	LT

Females WT (n = 10) **vs KI (**n = 10)

- Lactating MG (day=7)

SOCS2^{R96C} mice: size alteration

NanoScan PET/CT, Mediso
(*Service Exploration Non-Invasive, CREFRE*)