ImmGenATAC1219Sorts.S1A.pdf

Figure S1

A

Immunological Genome Project

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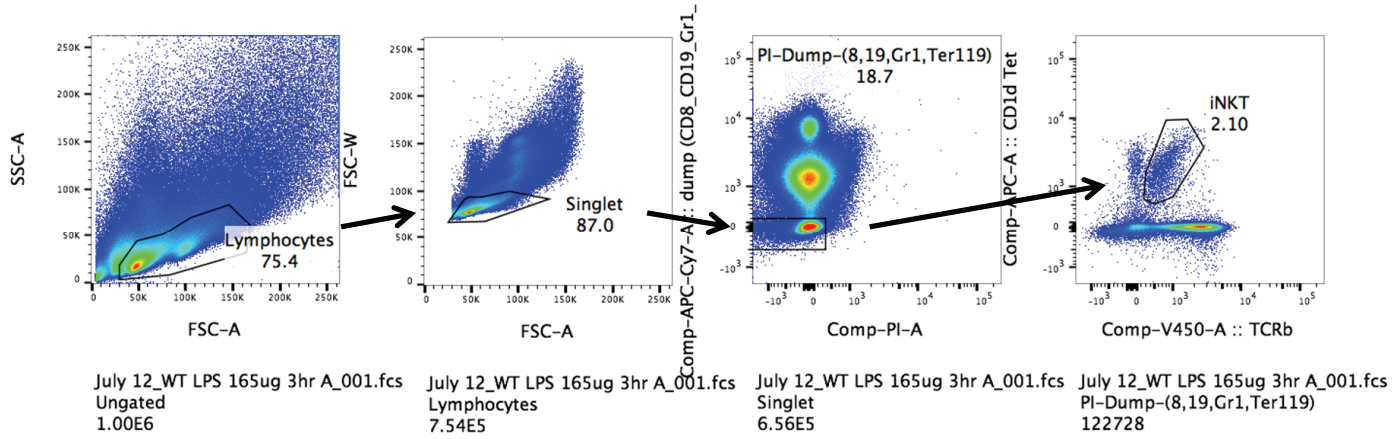
Edy Kim
Brenner Lab
Brigham & Women's Hospital

NKT.Sp.LPS.3hr: Splenic iNKT, 3h post-LPS challenge

Pre-sort



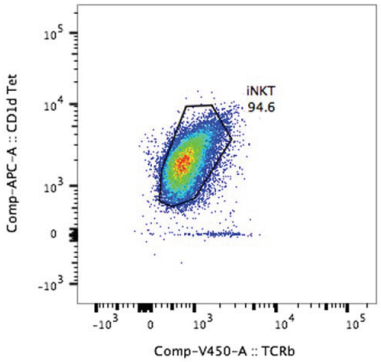
Post-1st sort

Tissue Preparation : Mechanical disruption of spleen

Time from Mouse to Trizol: 2.5 hours

Antibody (Clone) - Fluorochrome

TCRb (H57-597)—V450
CD8 (53-6.7), CD19 (1D3), Gr1 (8C5), Ter119 (Ter119) -- APC-Cy7
CD1d tetramer loaded with PBS-57 -- APC
(provided by NIH tetramer facility)



94.6 pure after 1st sort, >>95% pure on 2nd sort

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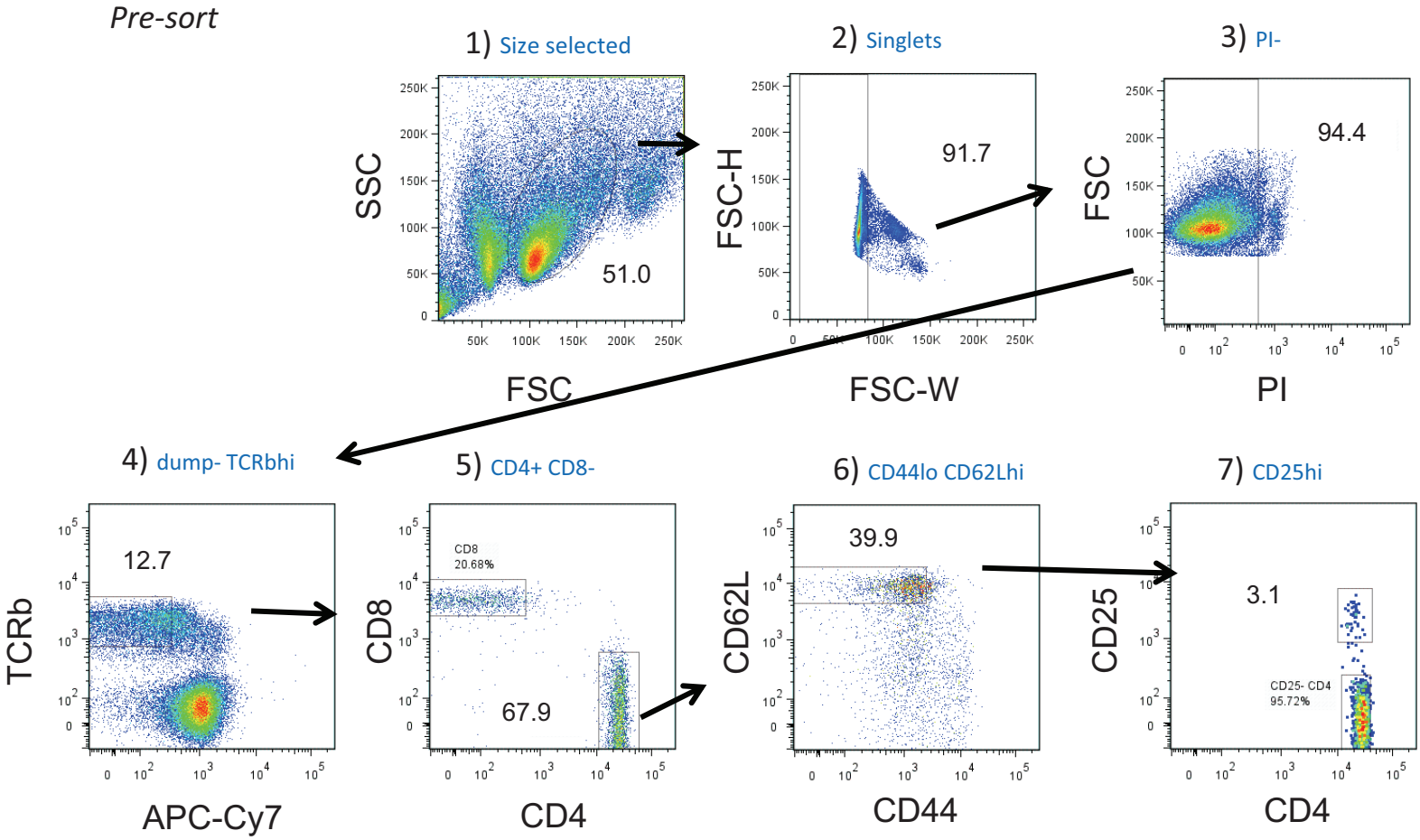
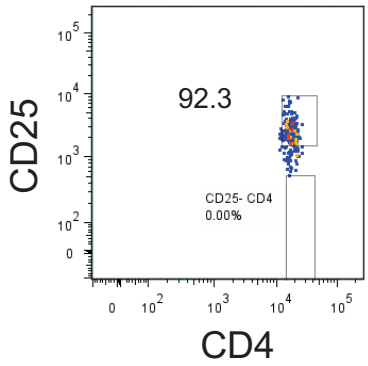
Treg.4.25hi.Sp: Splenic regulatory T cells

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis,

Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) – APC, CD8a (53-6.7) – A700, TCRb (H57-597) – eF450,
CD44(IM7) – FITC, CD62L(Mel-14) – PE-Cy7, CD25(PC61) -- PE
dump = CD19(1D3), Ter119(Ter-119) , Gr1(RB6-8C5) – APC-eF780
NK1.1(PK136), F4/80(BM8) -- APC-Cy7

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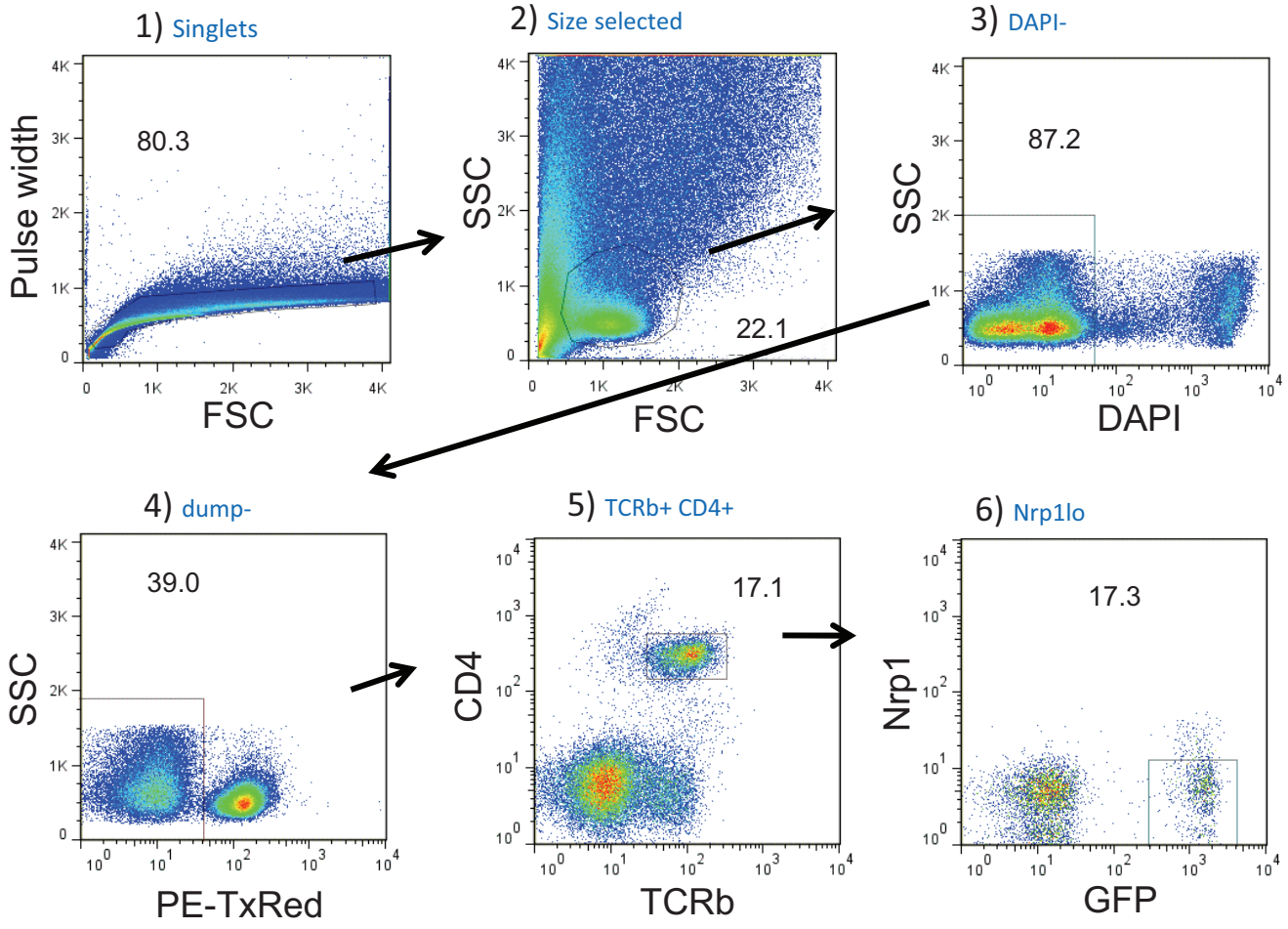
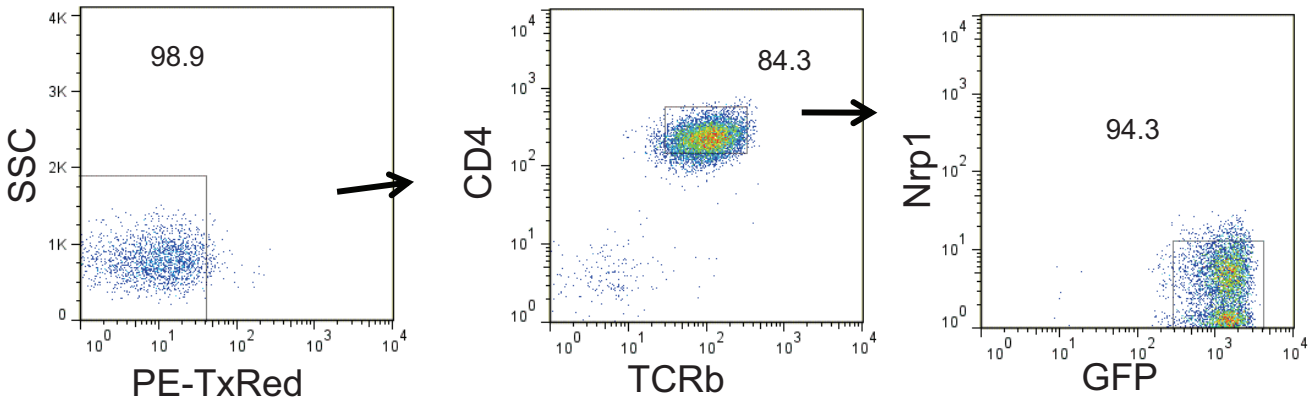
Treg.4.FP3+.Nrplo.Co: Colonic regulatory T cells

Pre-sort



2nd sorting



Tissue Preparation:
removing fat, stripping IEL layer and mucus by EDTA+DTT, digesting by dispase+collagenase

Time from Mouse to the 2nd sorting:
7am to 12am

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) – PE, TCRb (H57-597) – PE-Cy7, Foxp3-IRES-GFP,
Nrp1 -- APC
dump = CD19(1D3) – PE-TxRed

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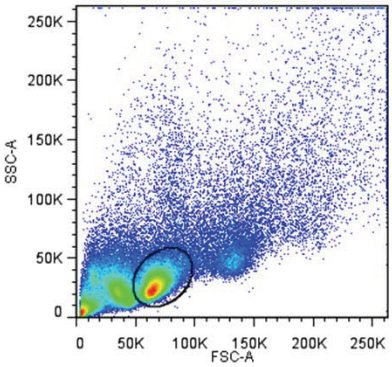
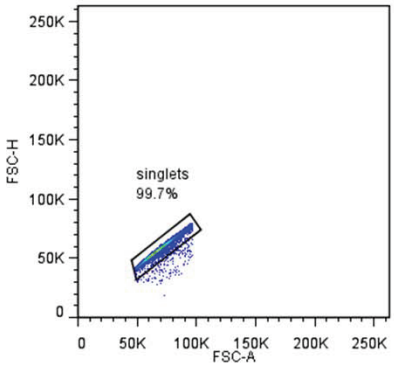
B.Fem.SP: Splenic B cell (Female)

Pre-sort

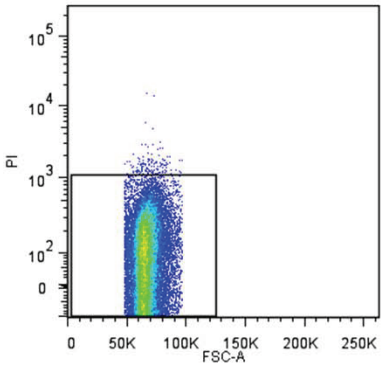
1) Lymphocytes



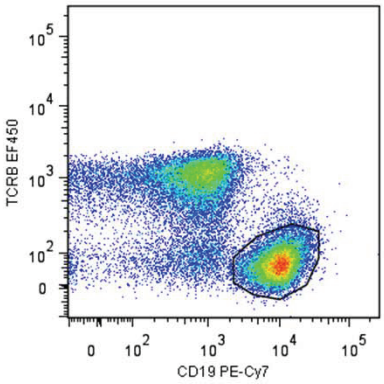
2) Singlets



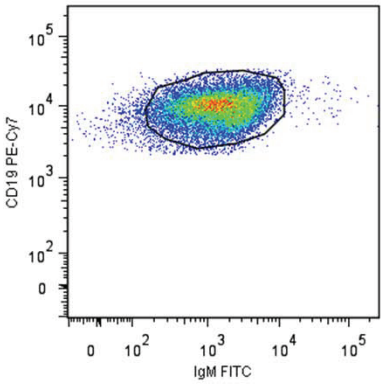
3) Live Cells



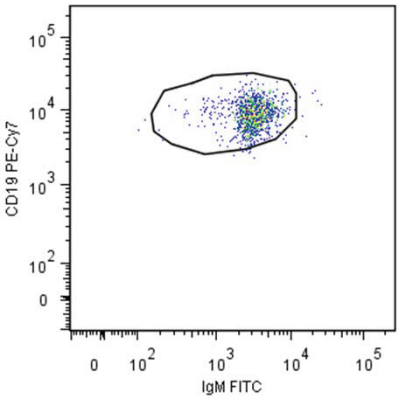
4) CD19+ Cells



6) B Cells



Post-sort



Tissue Preparation: Mechanical disruption of spleen
Time from Mouse to TCL Buffer: 10:30am-12:00pm

Antibody (Clone) - Fluorochrome:
TCRb (H57-597)—Pacific Blue
CD19 (1D3)—PE-Cy7
IgM (eB121-15F9)—FITC

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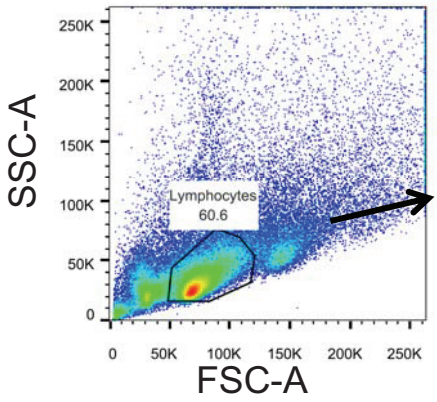
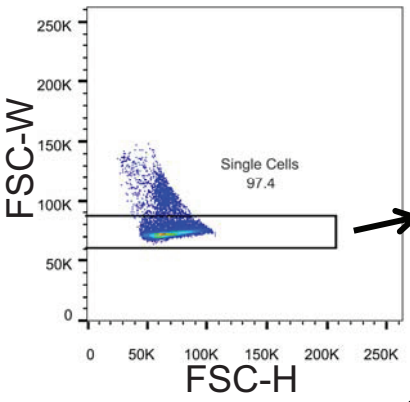
B.Sp: Splenic B cells

Pre-sort

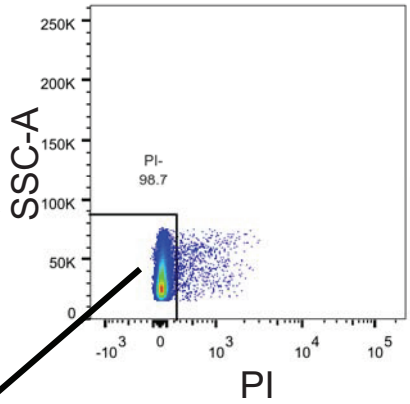
1) Size selected



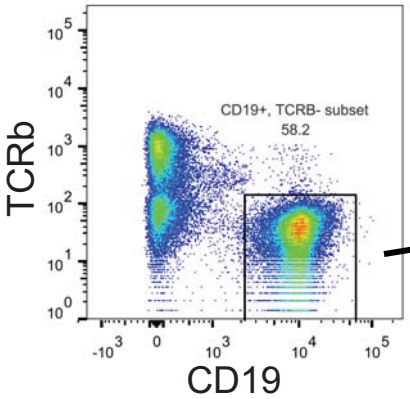
2) Singlets



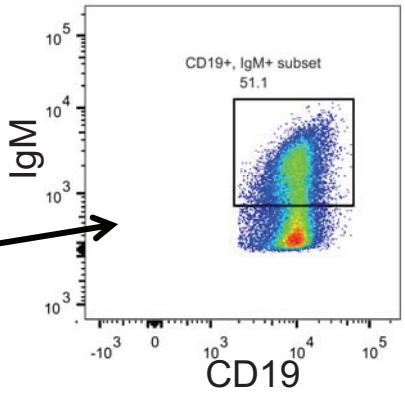
3) PI-



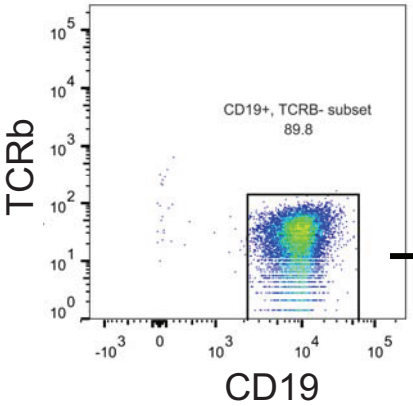
4) CD19+ TCRb-

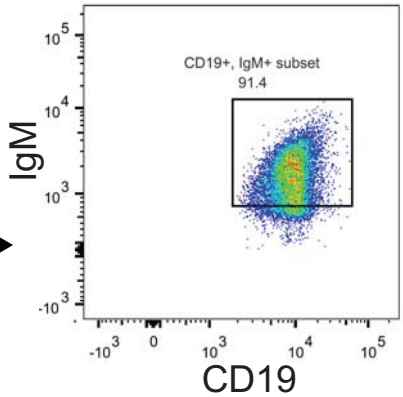


5) CD19+ IgM+



2nd sorting





Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis

Time from Mouse to the 2nd sorting:
9:30am to 11am

Antibody (Clone) - Fluorochrome:
TCRb(H57-597)--PB
CD19(1D3)-- PE-Cy7
IgM(eB121-15F9)--FITC

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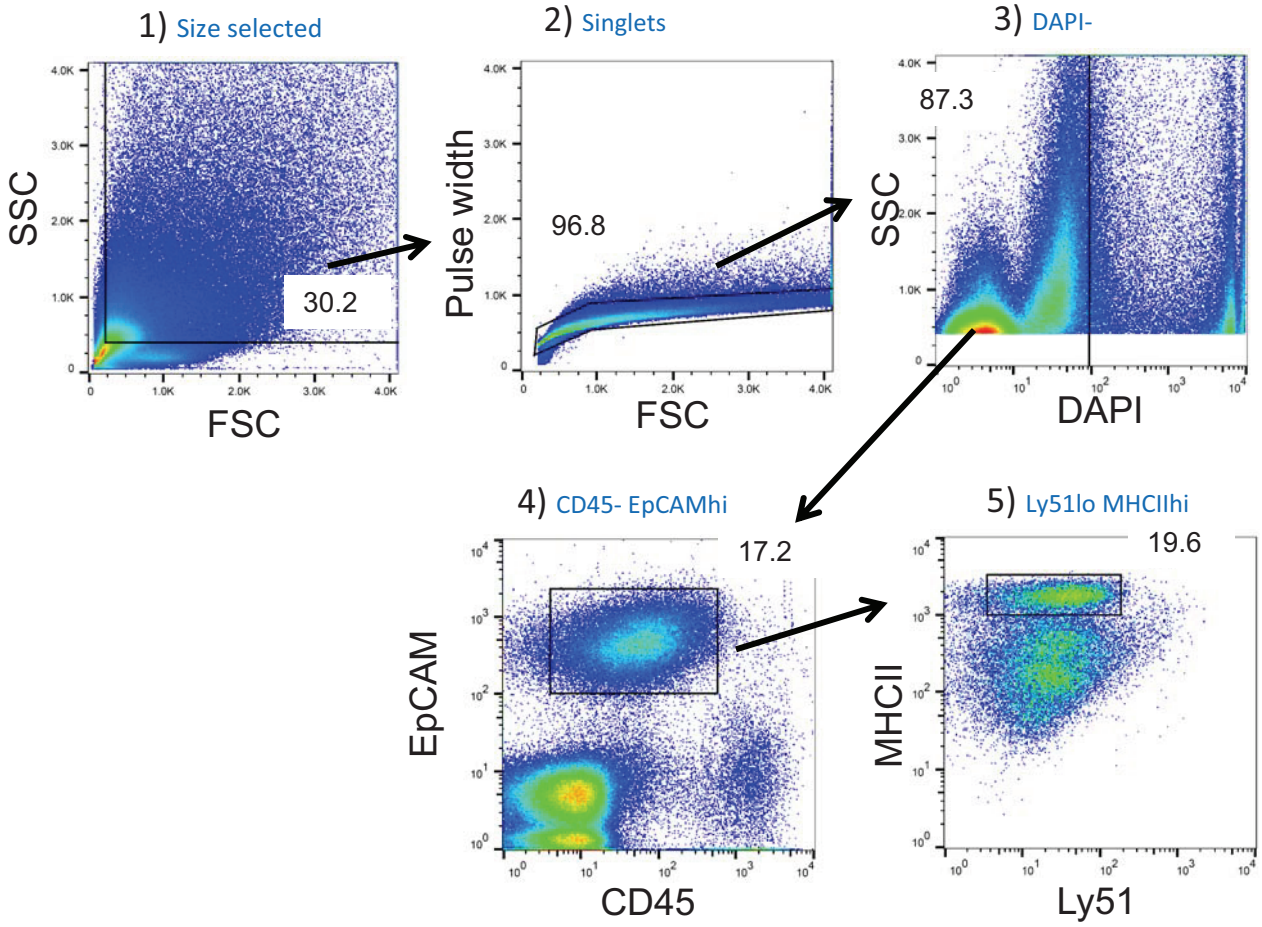
Ep.MECHi.Th: Thymic medullary epithelial cells

Pre-sort



Post-sort

Tissue Preparation:
Collagenase digestion, Collagenase/Dispase digestion, CD45 MACS depletion (LS column)

Time from Mouse to the 2nd sorting:
8am to 12am

Antibody (Clone) - Fluorochrome:
CD45 (30-F11) -- PE-CF594
Ly51 (6C3) -- PE
MHCII(M5/114) -- FITC
EpCAM(G8.8) -- APC

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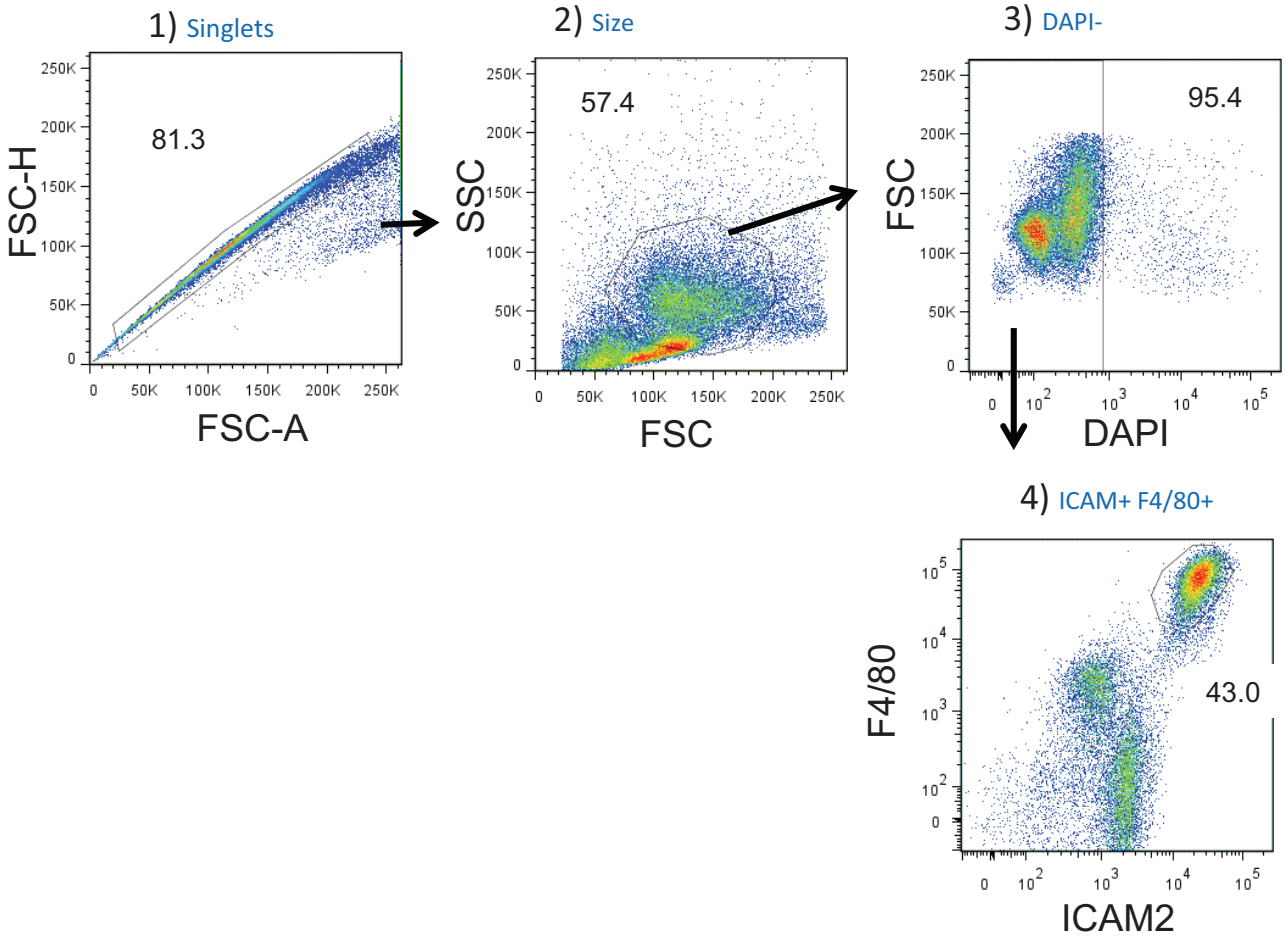
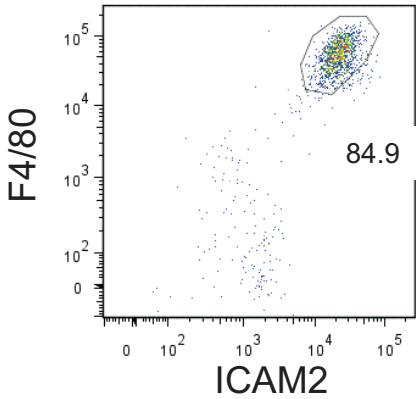
MF.Fem.PC: Peritoneal cavity macrophages (female)

Pre-sort



2nd sorting



Tissue Preparation:
Peritoneal lavage with Phenol-red free DMEM, ACK lysis

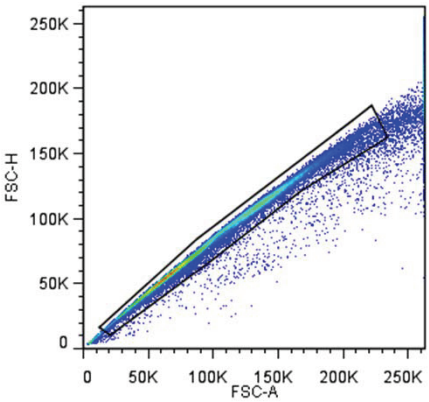
Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
F4/80 (BM8) -- PE-Cy7
ICAM2 (3C4 (mIC2/4)) -- FITC

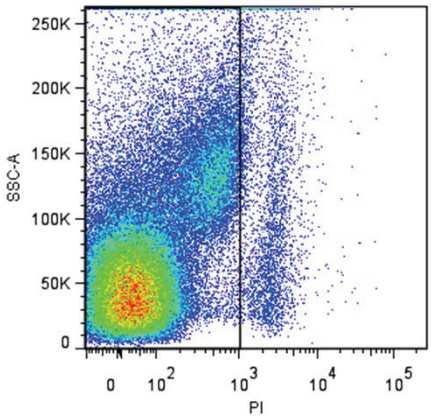
MF.PC: Peritoneal cavity macrophages

Pre-sort

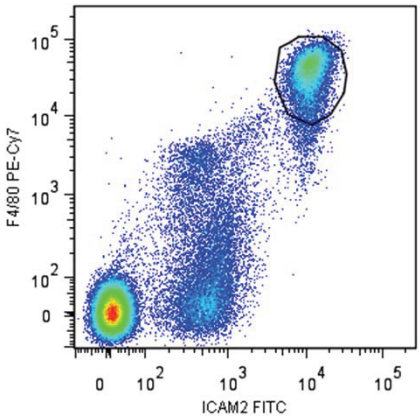
1) Single Cells



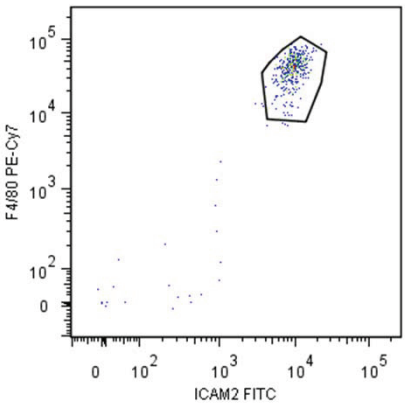
2) Live Cells



3) MF: F4/80+ ICAM2+



Post-sort



Tissue Preparation: Peritoneal Cavity Lavage

Time from Mouse to TCL Buffer: 8:30am-11:00am

Antibody (Clone) - Fluorochrome:

ICAM2 (mIC2/4)—FITC
F4/80 (BM8)—PE

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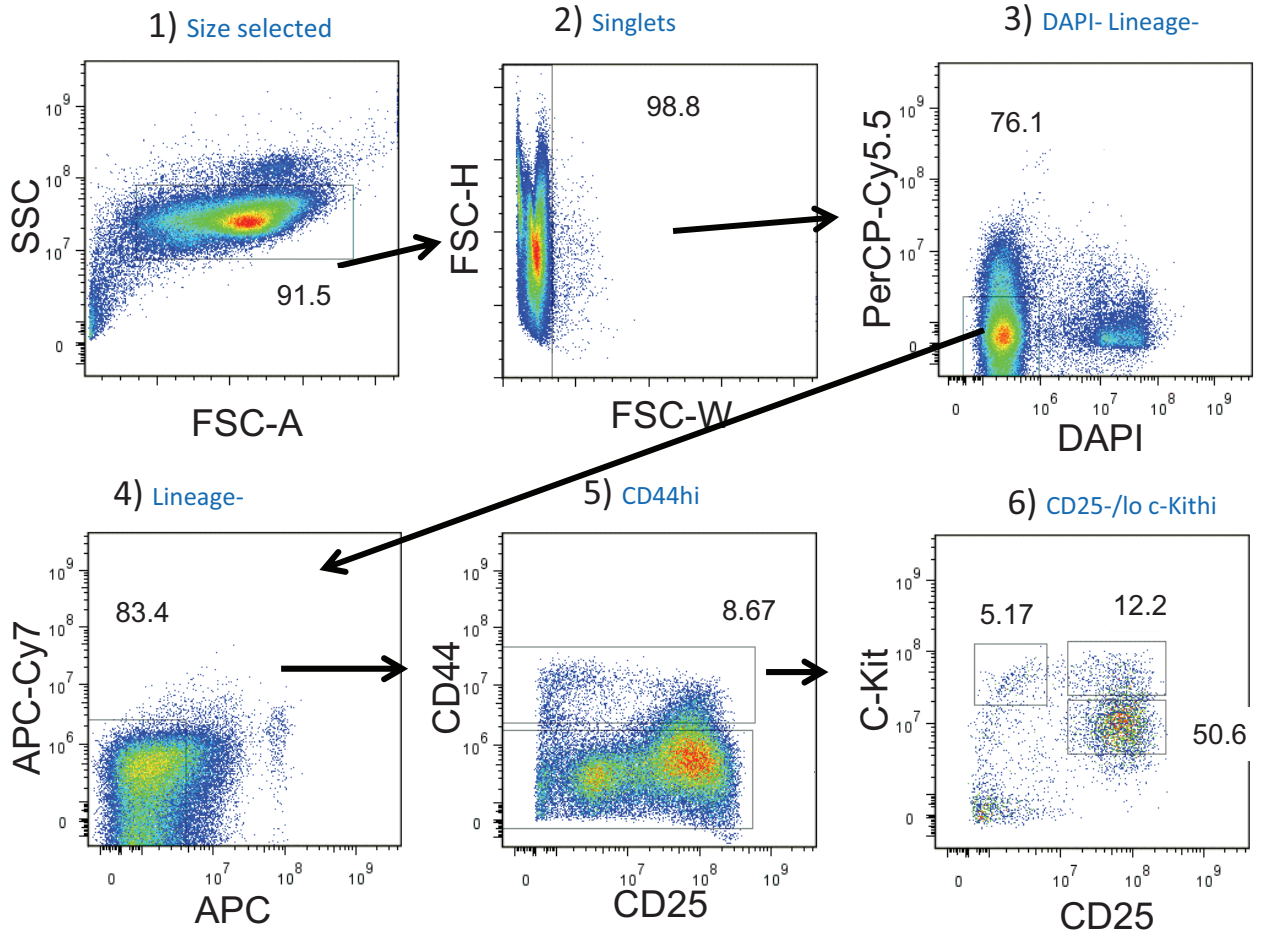
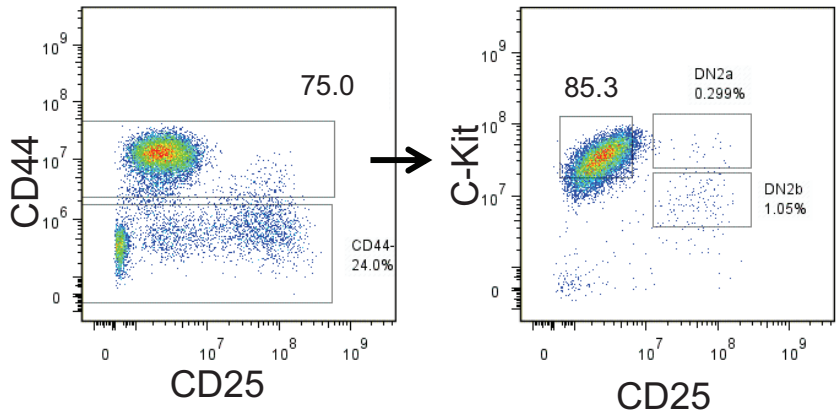
preT.DN1.Th: Pre-TCR DN1 thymocytes

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis, MACS depletion with APC-beads and LS column

Time from Mouse to the 2nd sorting:
8am to 11am

Antibody (Clone) - Fluorochrome:
CD25 (PC61) – PE, CD44 (IM7) – FITC
CD117 (2B8) – PE-Cy7
Lin = CD4(RM4-5), CD8a(53-6.7), NK1.1(PK136) – APC
CD11b(M1/70), TCRb(H57-597), TCRgd(GL3) – PerCP-Cy5.5
CD11c(N418), CD19(1D3),Ter119(Ter-119), Gr1(RB6-8C5) – APC-Cy7

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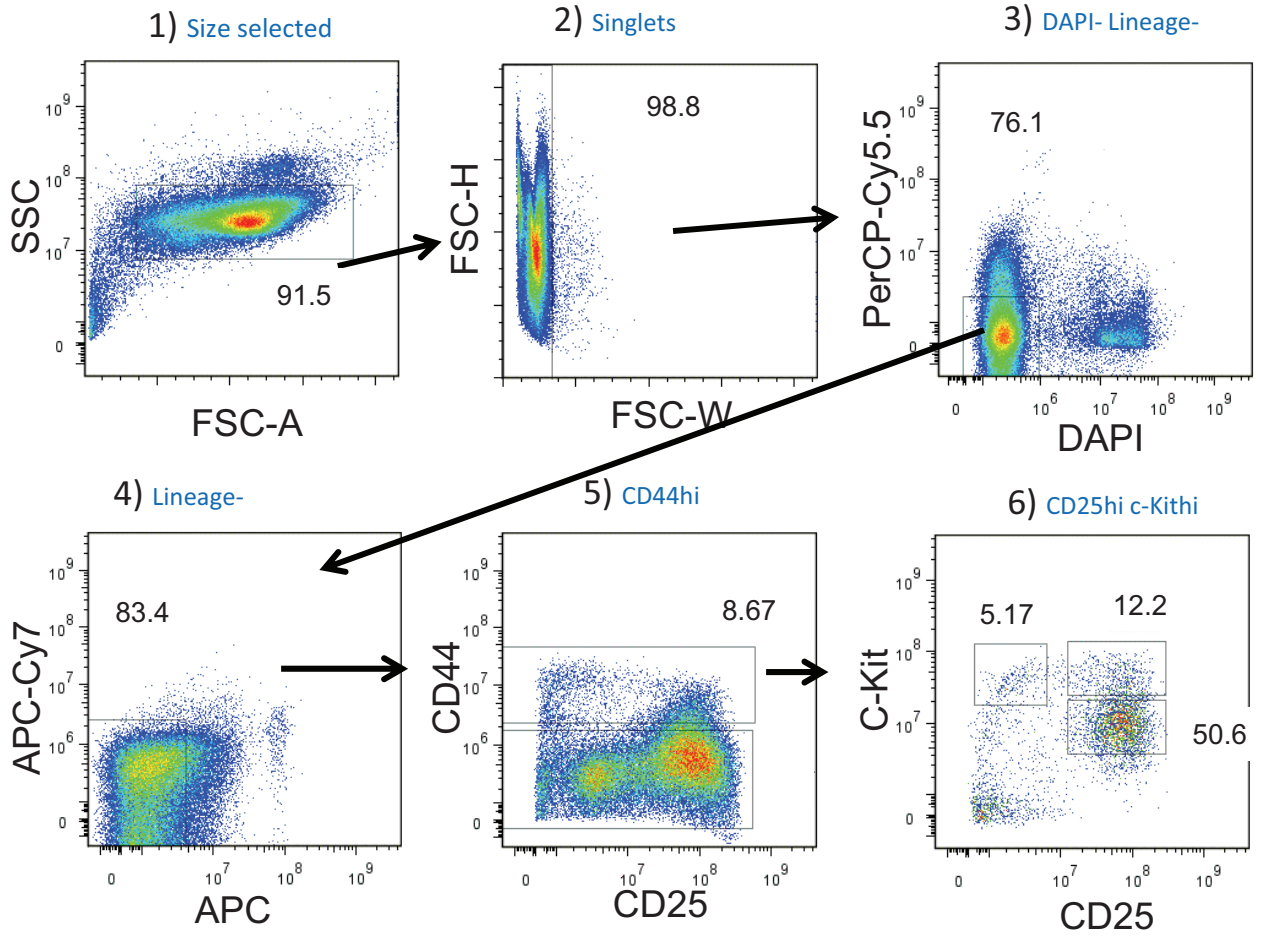
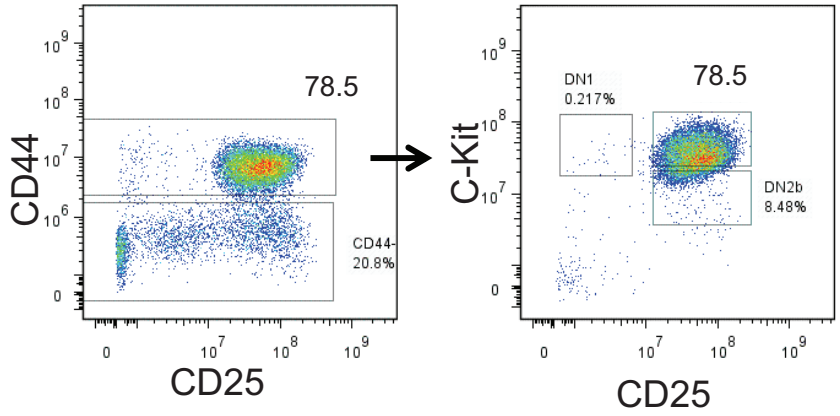
preT.DN2a.Th: Pre-TCR DN2a thymocytes

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis, MACS depletion with APC-beads and LS column

Time from Mouse to the 2nd sorting:
8am to 11am

Antibody (Clone) - Fluorochrome:
CD25 (PC61) – PE, CD44 (IM7) – FITC
CD117 (2B8) – PE-Cy7
Lin = CD4(RM4-5), CD8a(53-6.7), NK1.1(PK136) – APC
CD11b(M1/70), TCRb(H57-597), TCRgd(GL3) – PerCP-Cy5.5
CD11c(N418), CD19(1D3),Ter119(Ter-119), Gr1(RB6-8C5) – APC-Cy7

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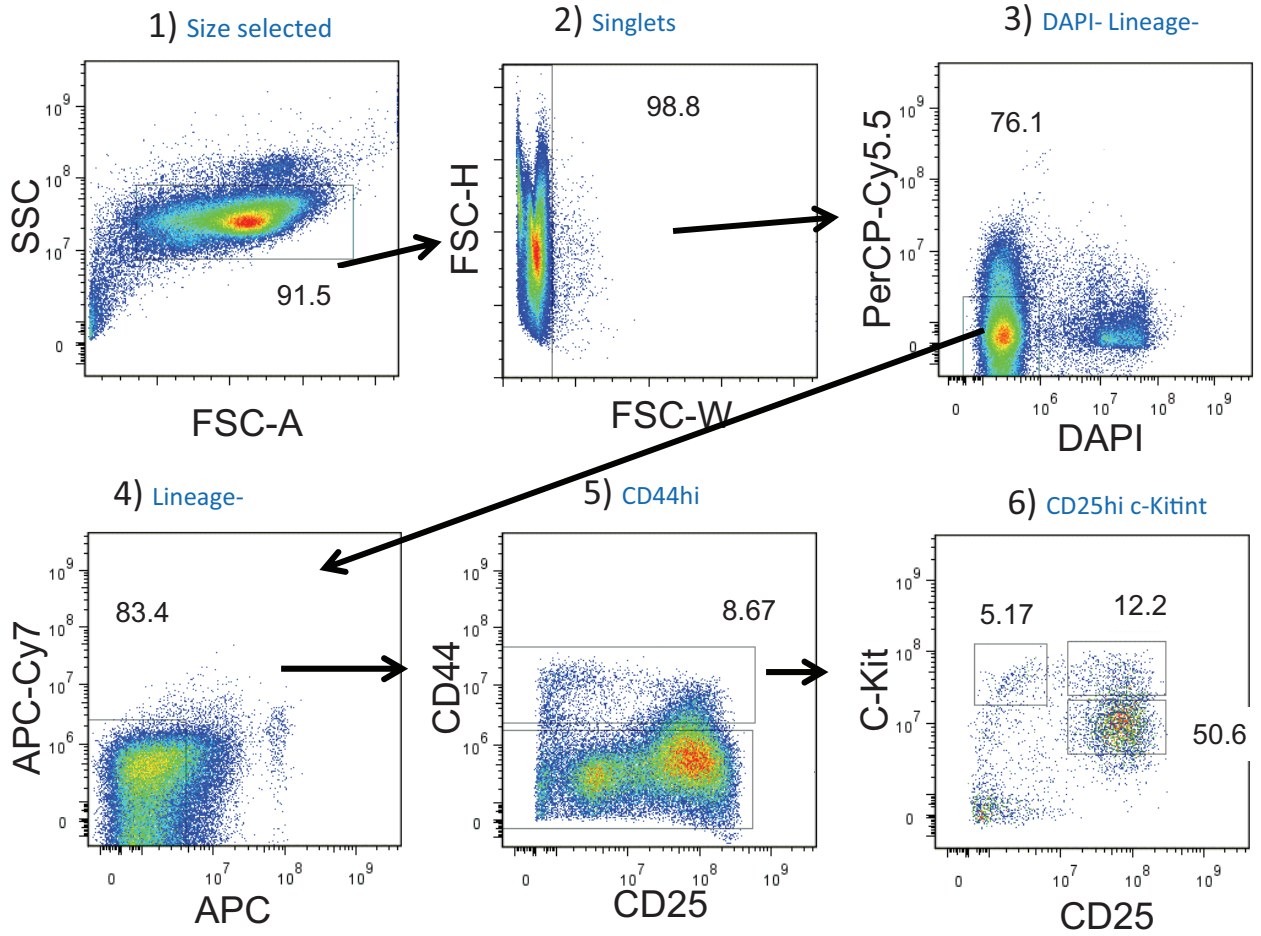
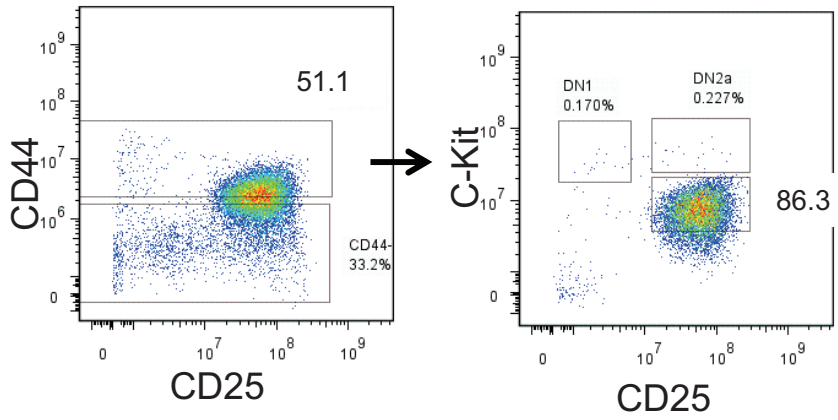
preT.DN2b.Th: Pre-TCR DN2b thymocytes

Pre-sort



2nd sorting



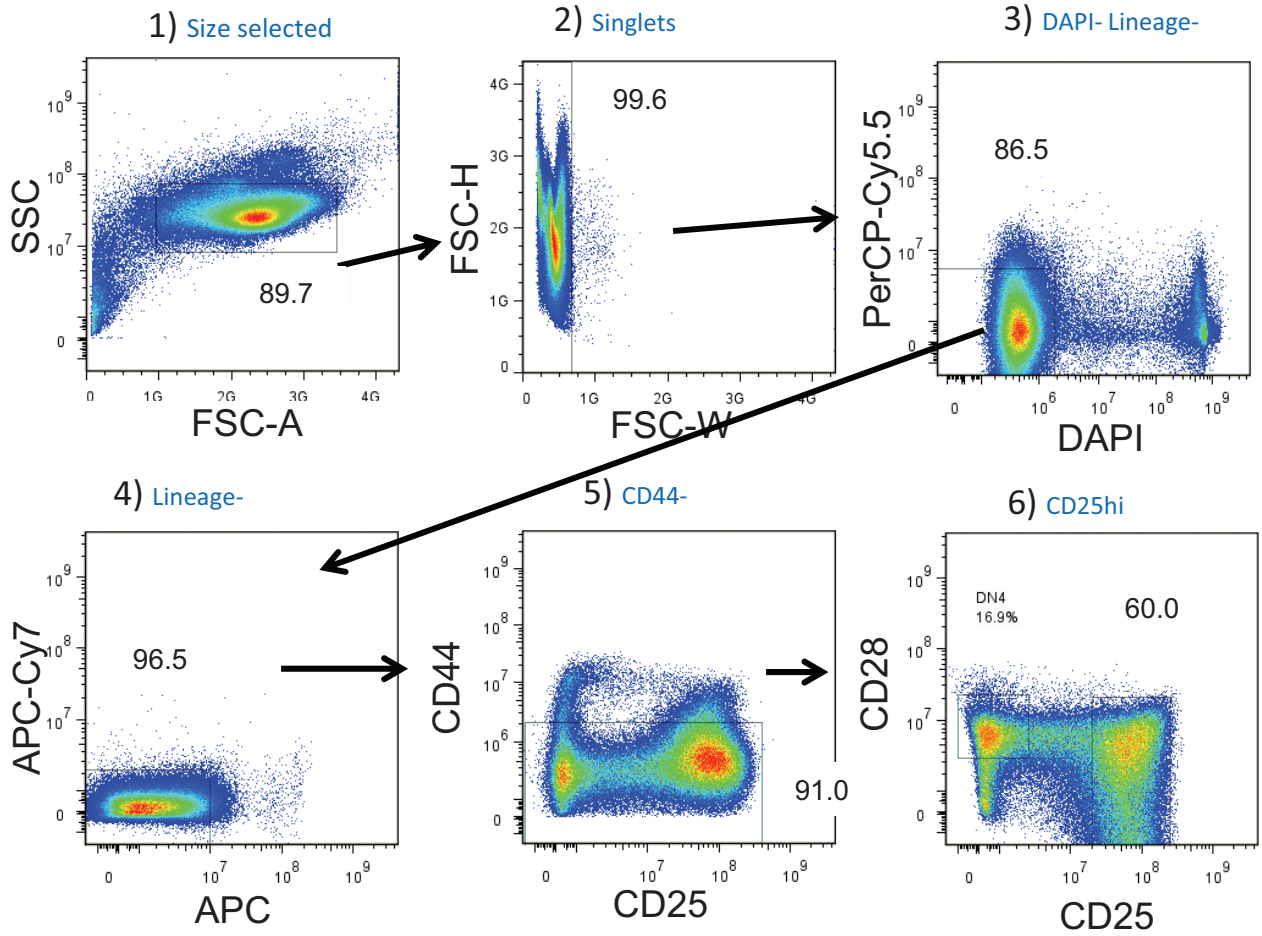
Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis, MACS depletion with APC-beads and LS column

Time from Mouse to the 2nd sorting:
8am to 11am

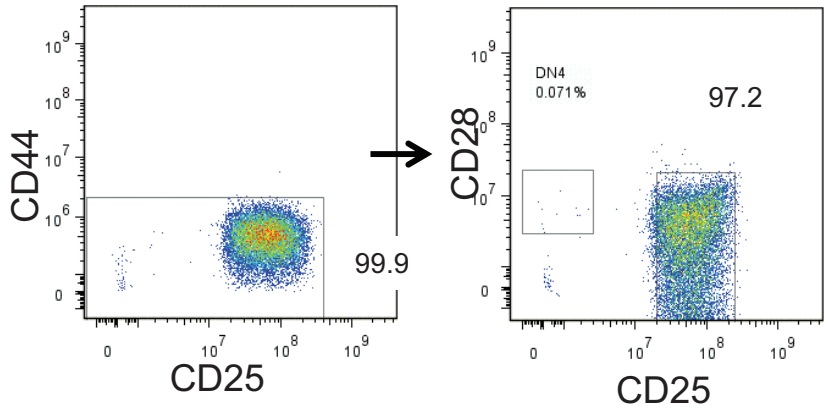
Antibody (Clone) - Fluorochrome:
CD25 (PC61) – PE, CD44 (IM7) – FITC
CD117 (2B8) – PE-Cy7
Lin = CD4(RM4-5), CD8a(53-6.7), NK1.1(PK136) – APC
CD11b(M1/70), TCRb(H57-597), TCRgd(GL3) – PerCP-Cy5.5
CD11c(N418), CD19(1D3),Ter119(Ter-119), Gr1(RB6-8C5) – APC-Cy7

preT.DN3.Th: Pre-TCR DN3 thymocytes

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis, MACS depletion with APC-beads and LS column

Time from Mouse to the 2nd sorting:
8am to 11am

Antibody (Clone) - Fluorochrome:
CD25 (PC61) – PE, CD44 (IM7) – FITC
CD117 (2B8) – PE-Cy7, CD28(E18) -bio-SA -- PETxRed
Lin = CD4(RM4-5), CD8a(53-6.7), NK1.1(PK136) – APC
CD11b(M1/70), TCRb(H57-597), TCRgd(GL3) – PerCP-Cy5.5
CD11c(N418), CD19(1D3),Ter119(Ter-119), Gr1(RB6-8C5) – APC-Cy7

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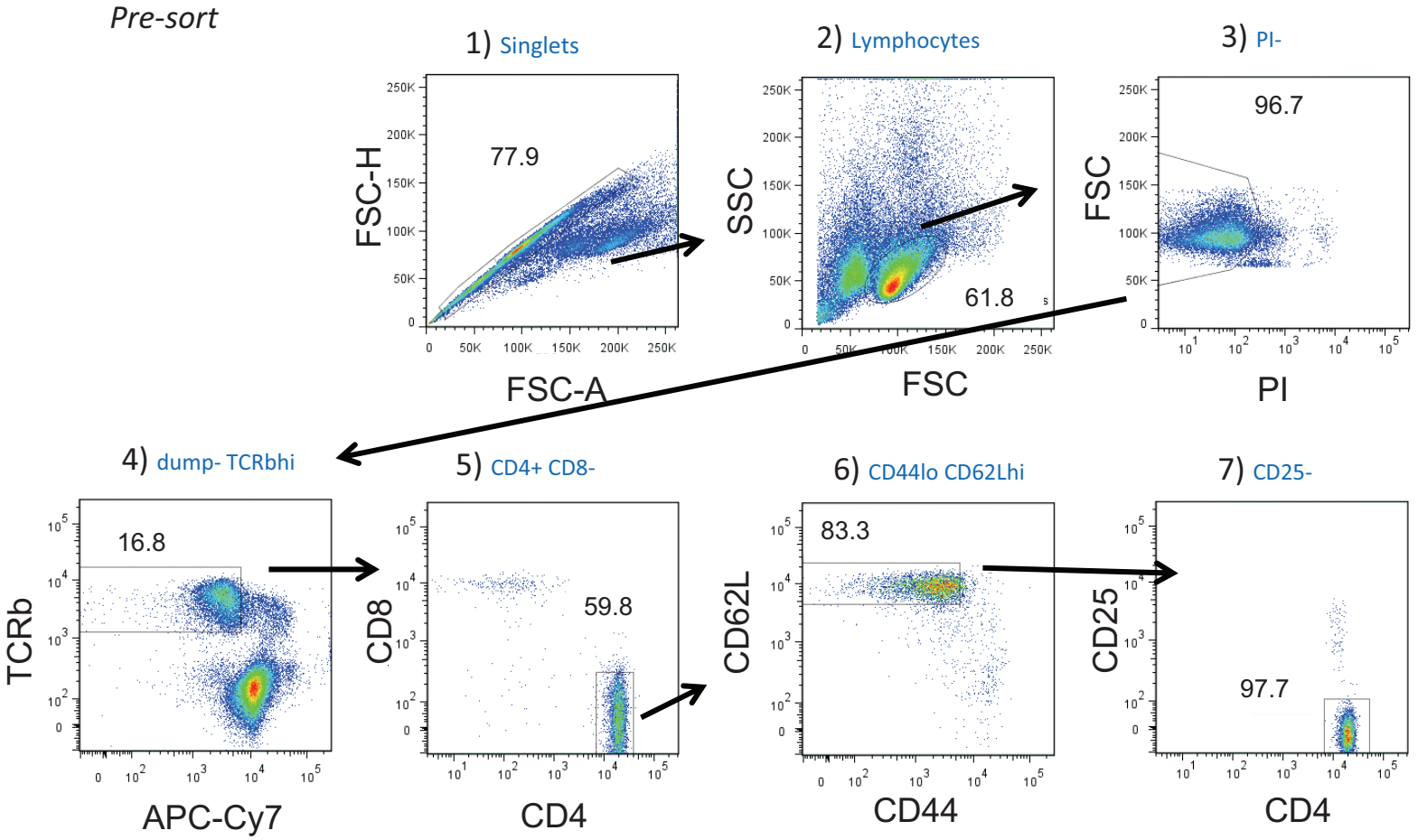
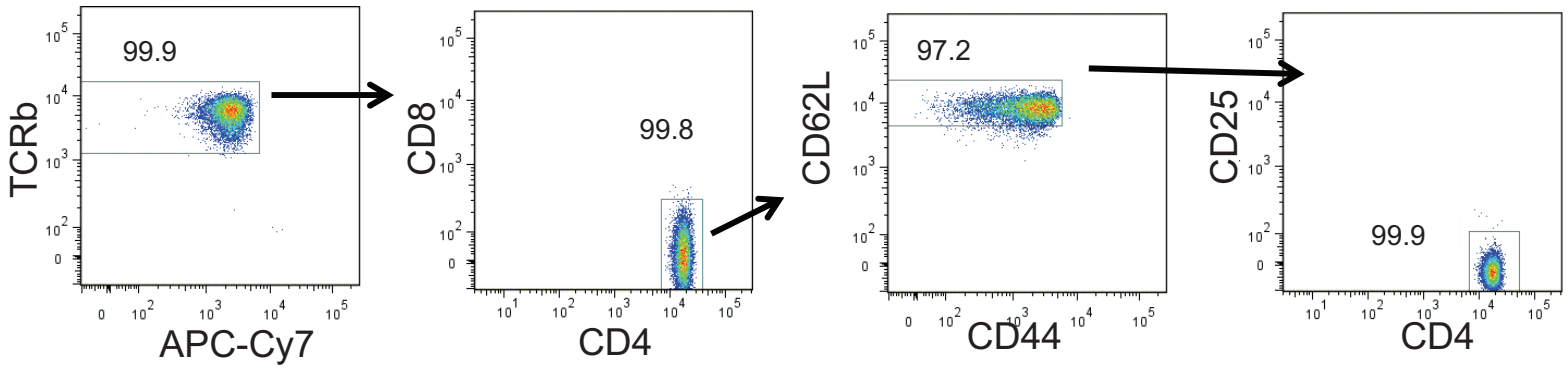
T.4.Nve.Fem.Sp: Splenic Naïve CD4 T cells (female)

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis

Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) – PE, CD8a (53-6.7) – A700, TCRb (H57-597) – eF450,
CD44(IM7) – FITC, CD62L(Mel-14) – PE-Cy7, CD25(PC61) -- APC
dump = CD19(1D3), Ter119(Ter-119) , Gr1(RB6-8C5) – APC-eF780
NK1.1(PK136), F4/80(BM8) -- APC-Cy7

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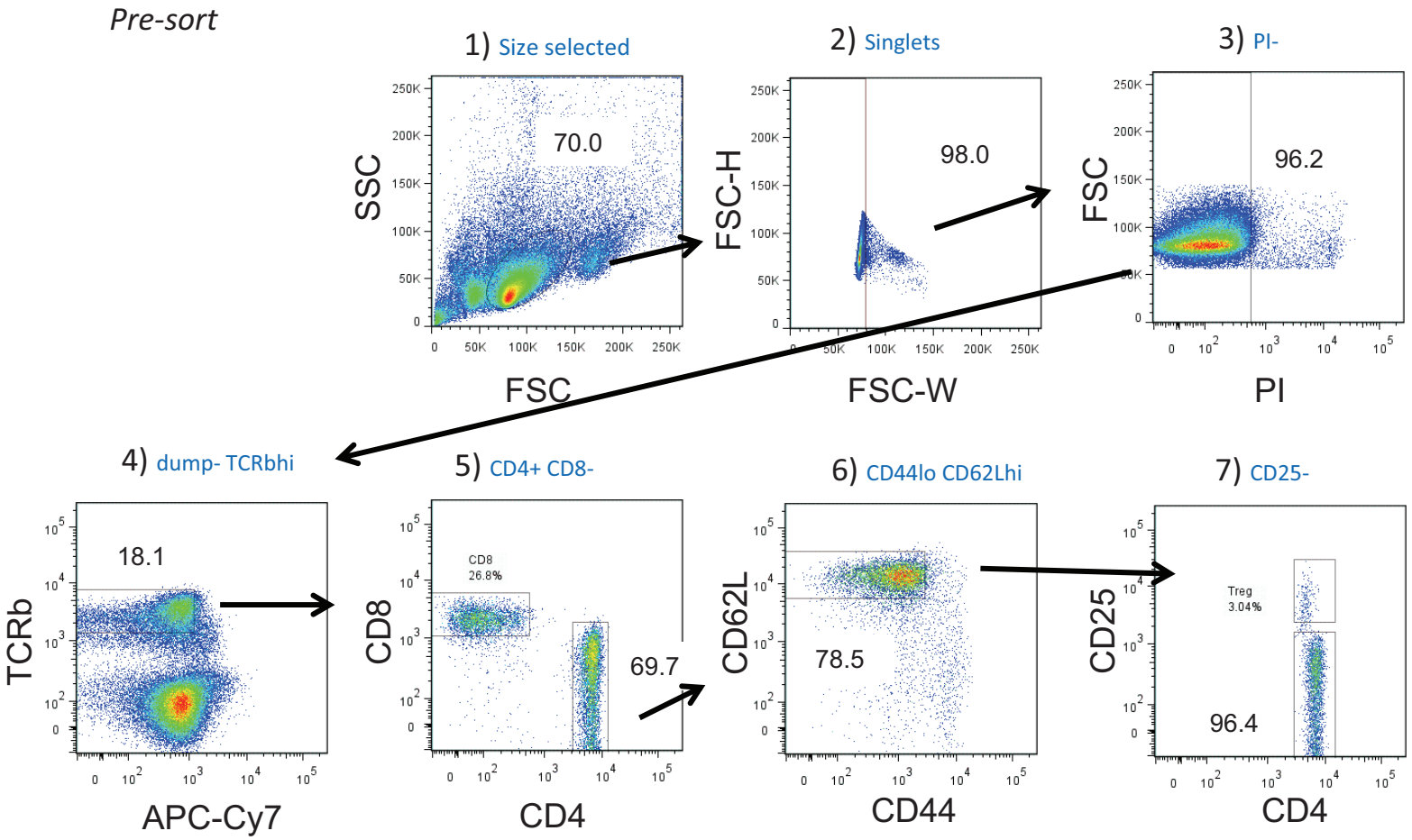
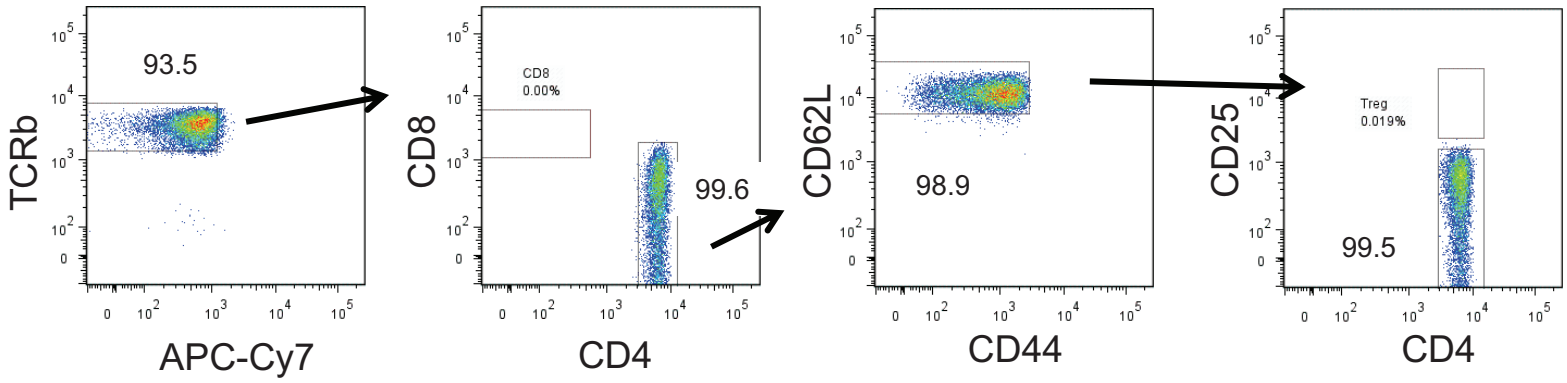
T.4.Nve.Sp: Splenic Naïve CD4 T cells

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis,

Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) – APC, CD8a (53-6.7) – A700, TCRb (H57-597) – eF450,
CD44(IM7) – FITC, CD62L(Mel-14) – PE-Cy7, CD25(PC61) -- PE
dump = CD19(1D3), Ter119(Ter-119) , Gr1(RB6-8C5) – APC-eF780
NK1.1(PK136), F4/80(BM8) -- APC-Cy7

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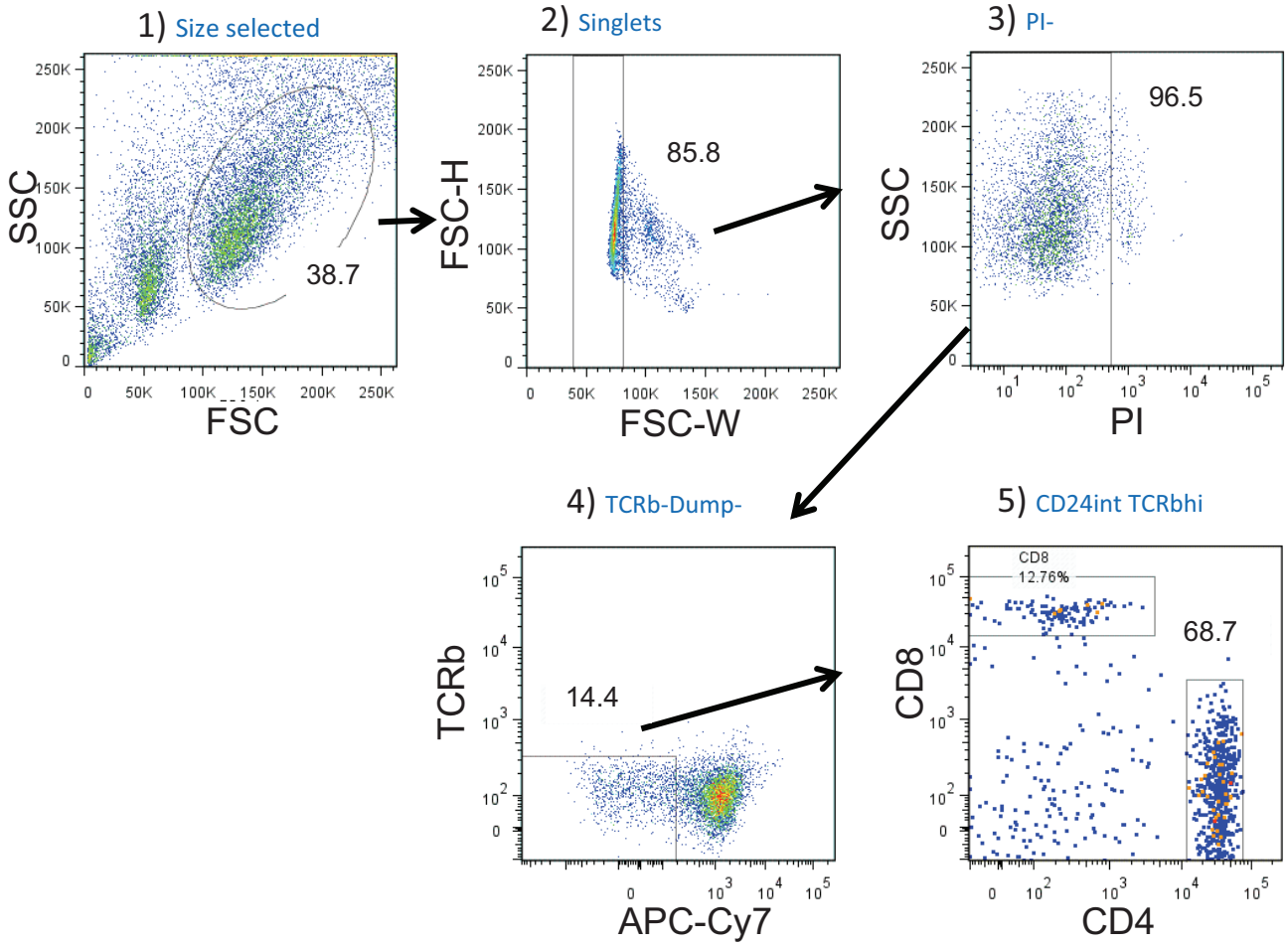
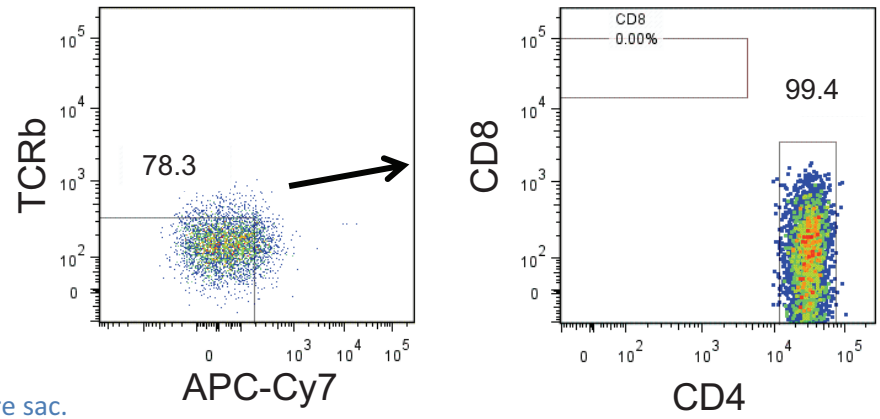
T.4.Sp.aCD3+CD40.18hr: Splenic CD4 T cells, 18hrs post anti-CD3+CD40

Pre-sort



2nd sorting



Tissue Preparation:
ip 60ug CD3(KT3) and 100ug CD40(FGK4.5) 18hrs before sac.
100um mesh/syringe plunger teasing, ACK lysis

Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) – PE, CD8a (53-6.7) – PE-Cy7, TCRb (H57-597) – eF450
dump = CD19(1D3), Ter119(Ter-119) , Gr1(RB6-8C5) – APC-eF780
NK1.1(PK136), F4/80(BM8) -- APC-Cy7

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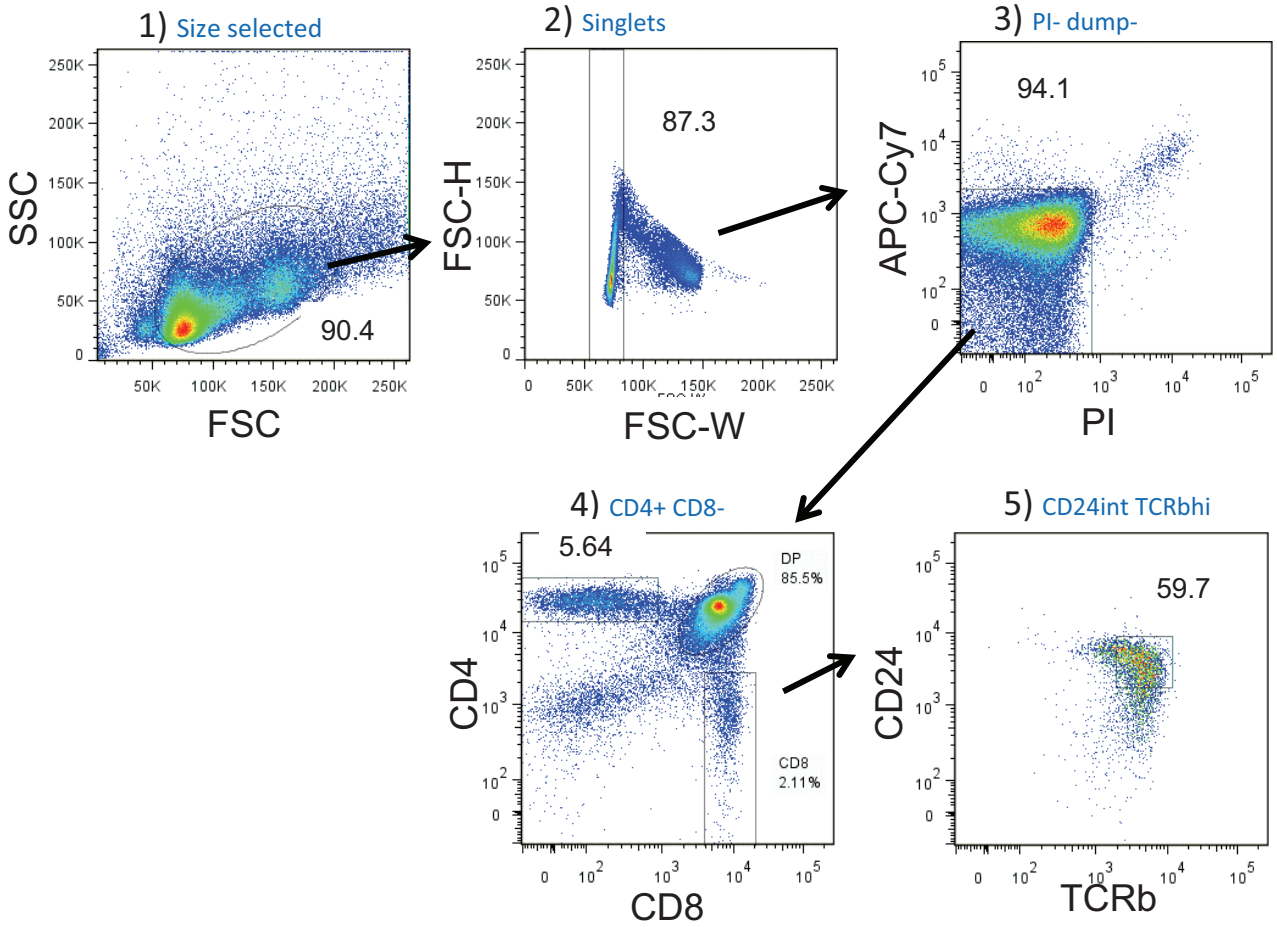
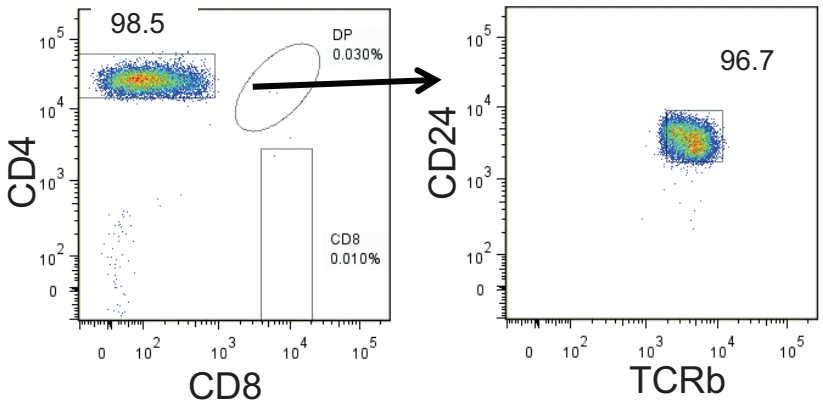
Harvard Medical School

T.4.Th: Thymic CD4SP T cells

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis,

Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) – PE, CD8a (53-6.7) – APC, TCRb (H57-597) – PB,
CD24(M1/69) -- FITC
dump = CD19(1D3), Ter119(Ter-119) , Gr1(RB6-8C5) – APC-eF780
NK1.1(PK136), F4/80(BM8) -- APC-Cy7

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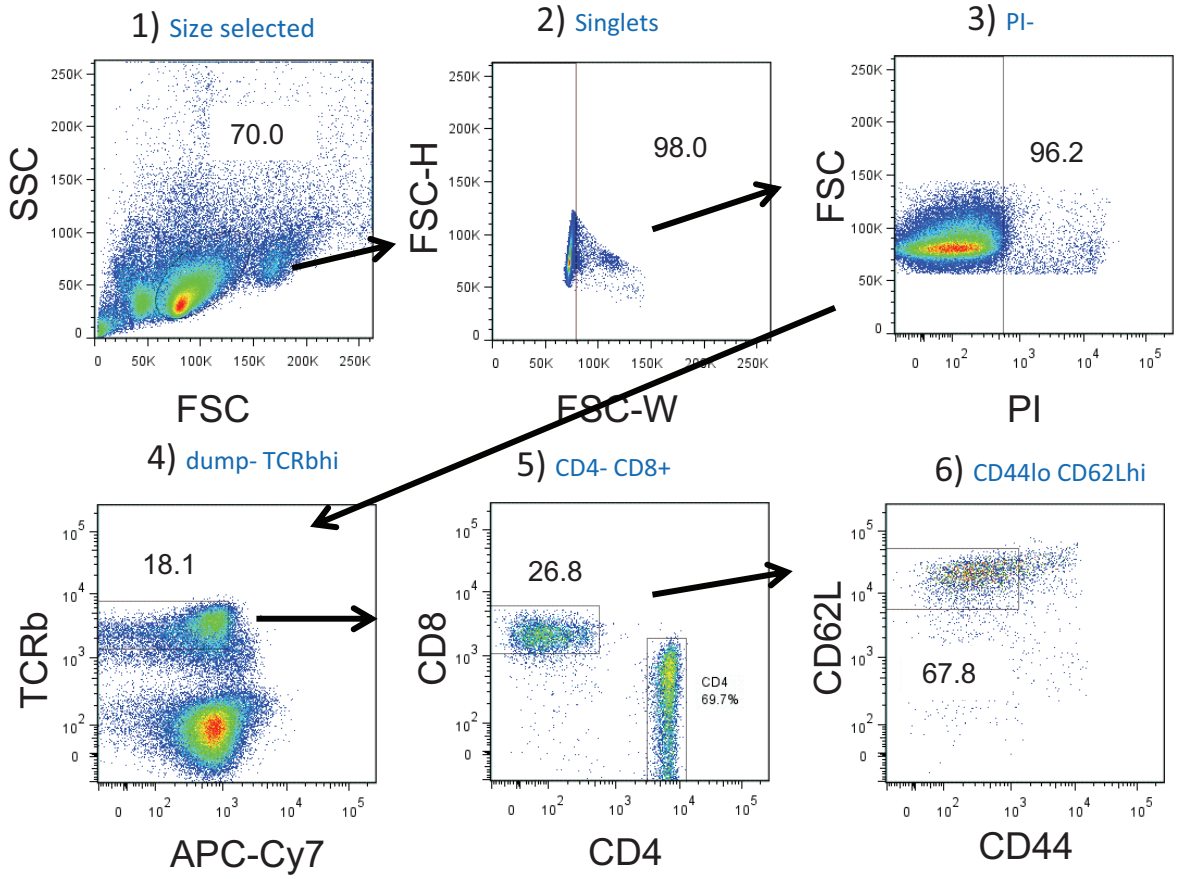
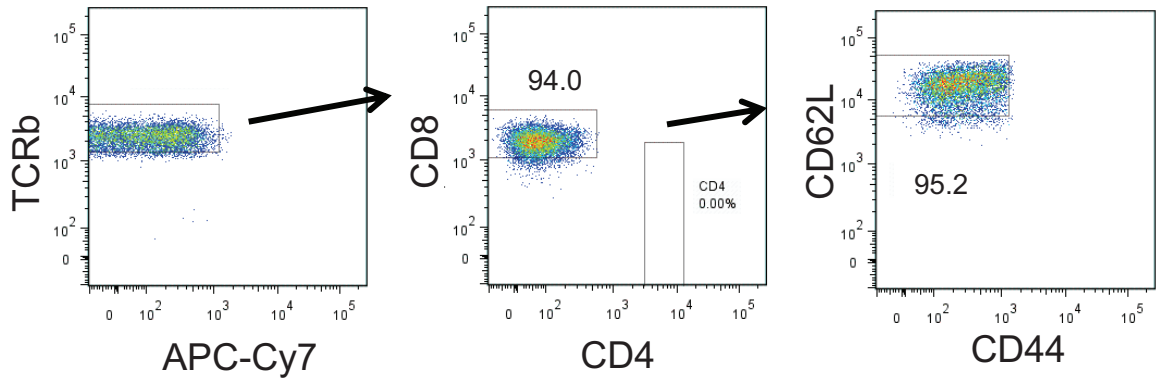
T.8.Nve.Sp: Splenic Naïve CD8 T cells

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis,

Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) – APC, CD8a (53-6.7) – A700, TCRb (H57-597) – eF450,
CD44(IM7) – FITC, CD62L(Mel-14) – PE-Cy7
dump = CD19(1D3), Ter119(Ter-119) , Gr1(RB6-8C5) – APC-eF780
NK1.1(PK136), F4/80(BM8) -- APC-Cy7

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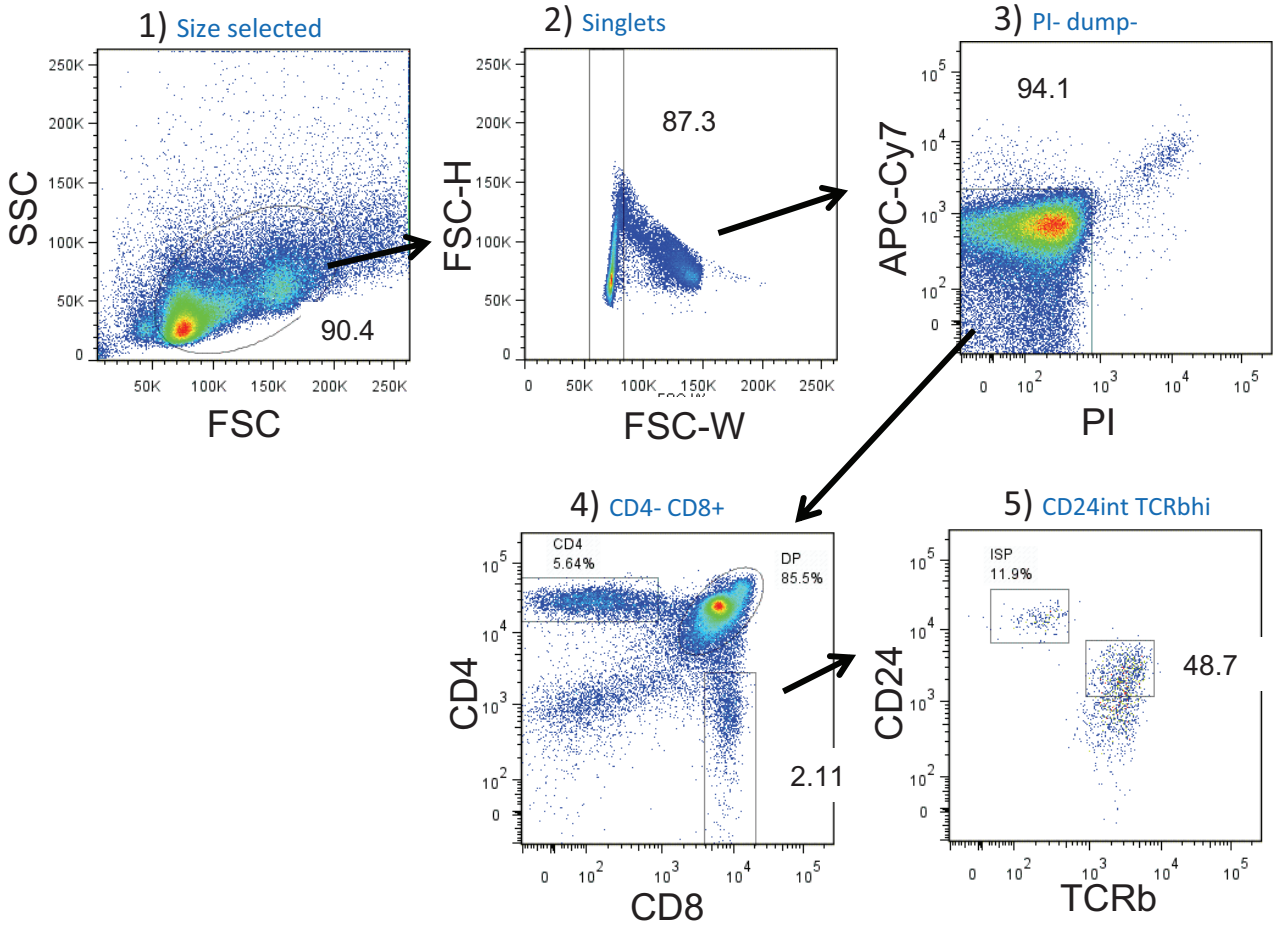
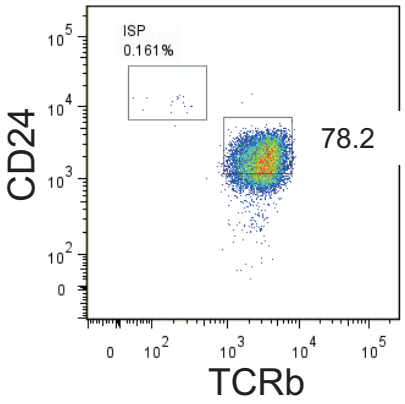
Harvard Medical School

T.8.Th: Thymic CD8SP T cells

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis,

Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) – PE, CD8a (53-6.7) – APC, TCRb (H57-597) – PB,
CD24(M1/69) -- FITC
dump = CD19(1D3), Ter119(Ter-119) , Gr1(RB6-8C5) – APC-eF780
NK1.1(PK136), F4/80(BM8) -- APC-Cy7

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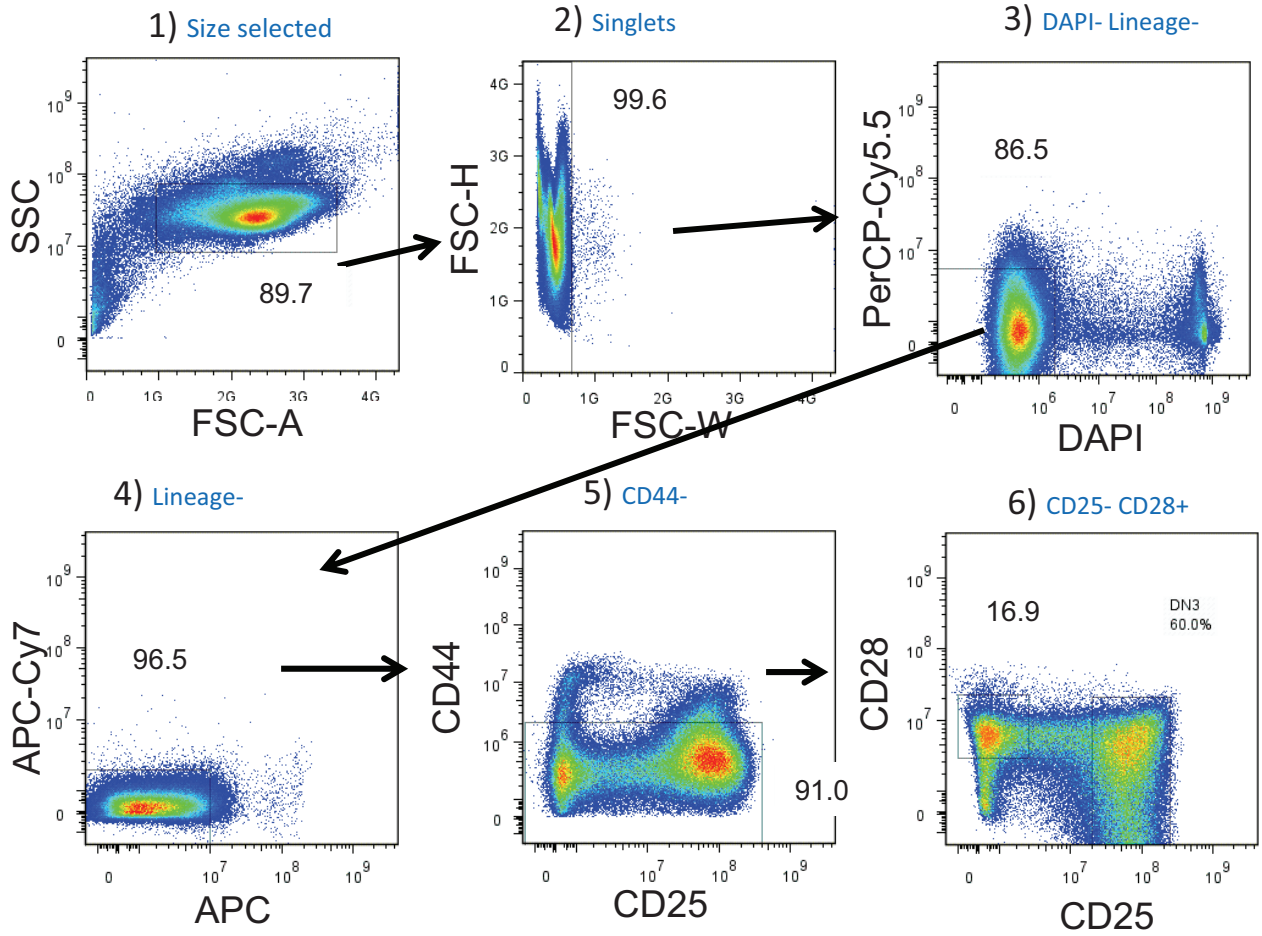
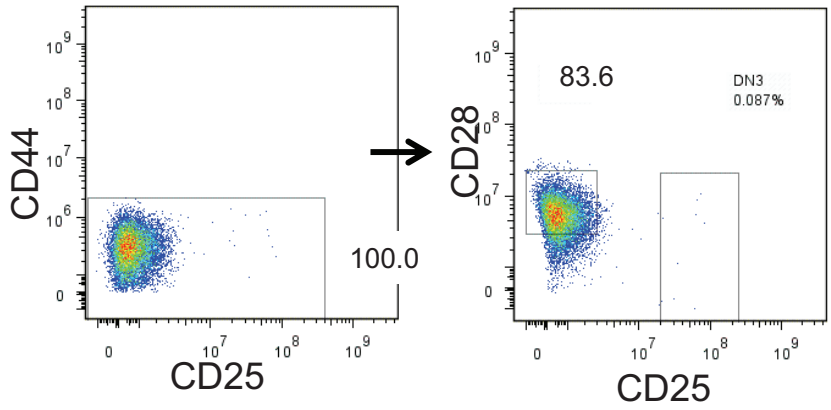
T.DN4.Th: DN4 thymocytes

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis, MACS depletion with APC-beads and LS column

Time from Mouse to the 2nd sorting:
8am to 11am

Antibody (Clone) - Fluorochrome:
CD25 (PC61) – PE, CD44 (IM7) – FITC
CD117 (2B8?) – PE-Cy7, CD28(E18) -bio-SA -- PETxRed
Lin = CD4(RM4-5), CD8a(53-6.7), NK1.1(PK136) – APC
CD11b(M1/70), TCRb(H57-597), TCRgd(GL3) – PerCP-Cy5.5
CD11c(N418), CD19(1D3),Ter119(Ter-119), Gr1(RB6-8C5) – APC-Cy7

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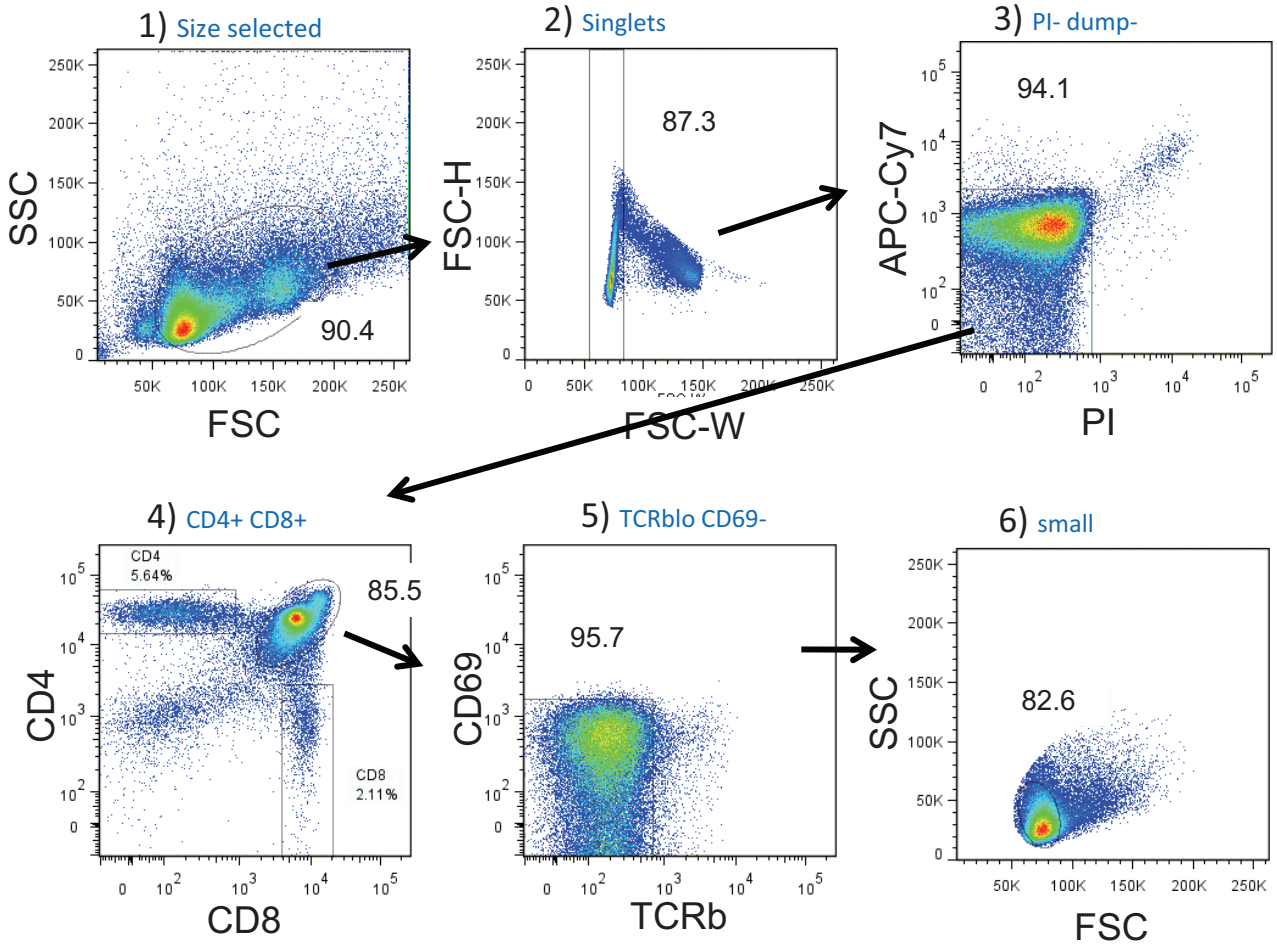
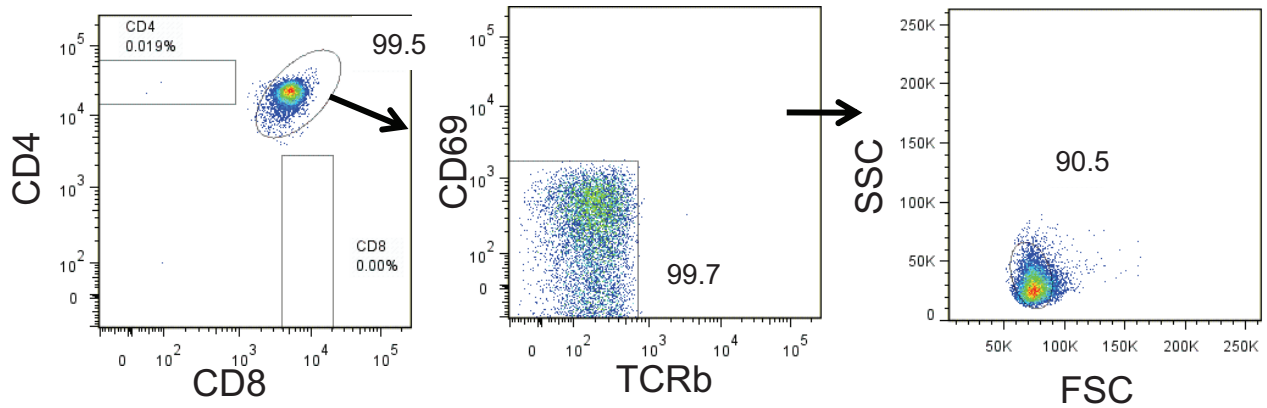
T.DP.Th: CD4+ CD8+ double-positive thymocytes

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis,

Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) – PE, CD8a (53-6.7) – APC, TCRb (H57-597) – PB,
CD24(M1/69) – FITC, CD69(H1.2F3) – A700
dump = CD19(1D3), Ter119(Ter-119) , Gr1(RB6-8C5) – APC-eF780
NK1.1(PK136), F4/80(BM8) -- APC-Cy7

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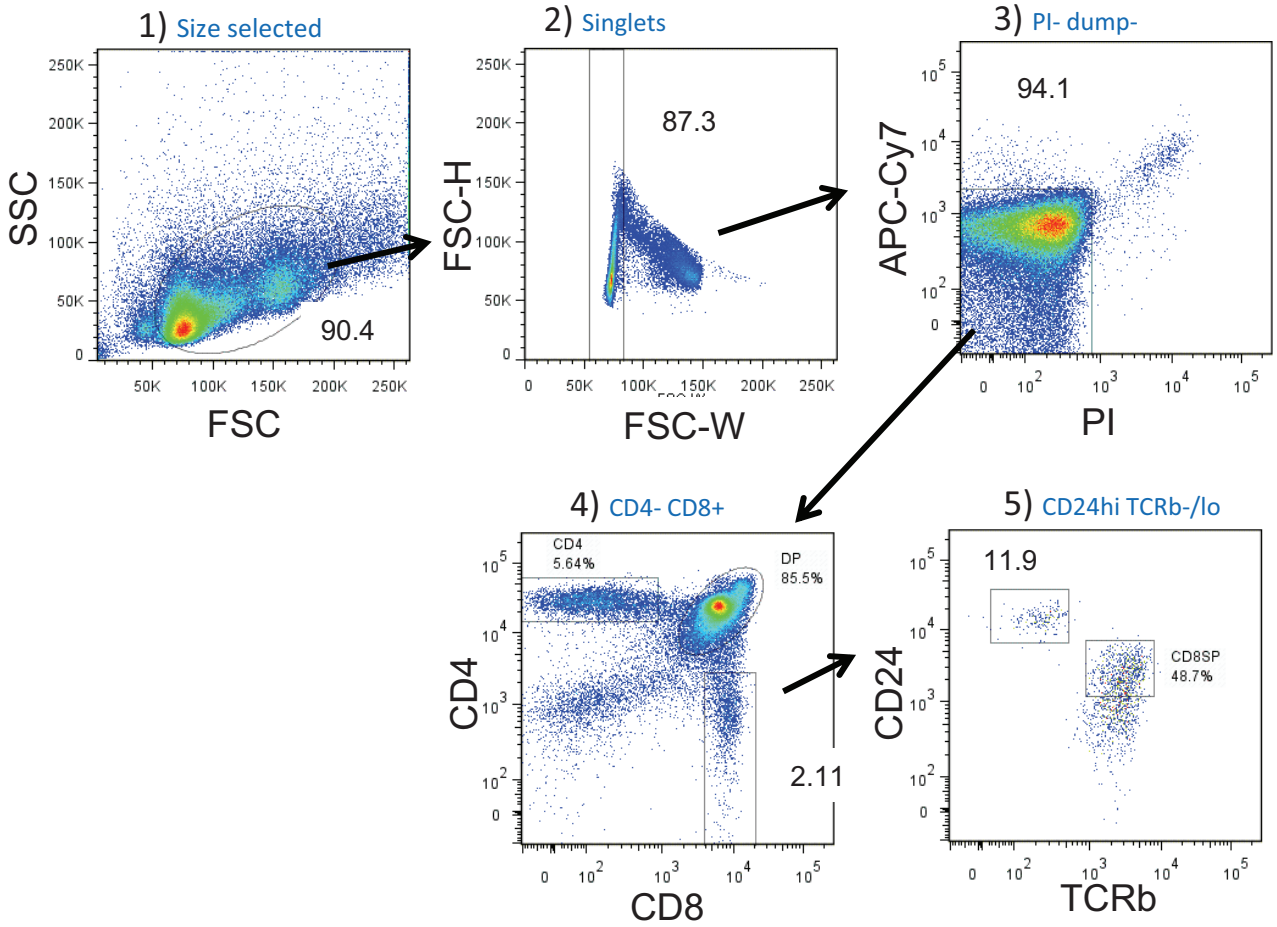
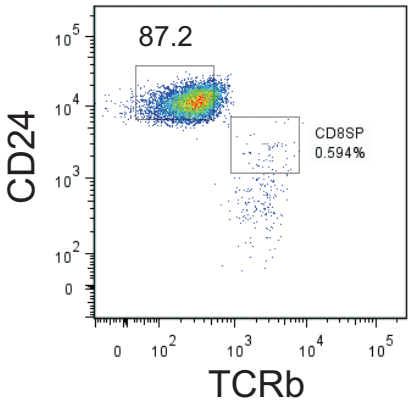
T.ISP.Th: Immature single-positive thymocytes

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis,

Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) – PE, CD8a (53-6.7) – APC, TCRb (H57-597) – PB,
CD24(M1/69) -- FITC
dump = CD19(1D3), Ter119(Ter-119) , Gr1(RB6-8C5) – APC-eF780
NK1.1(PK136), F4/80(BM8) -- APC-Cy7

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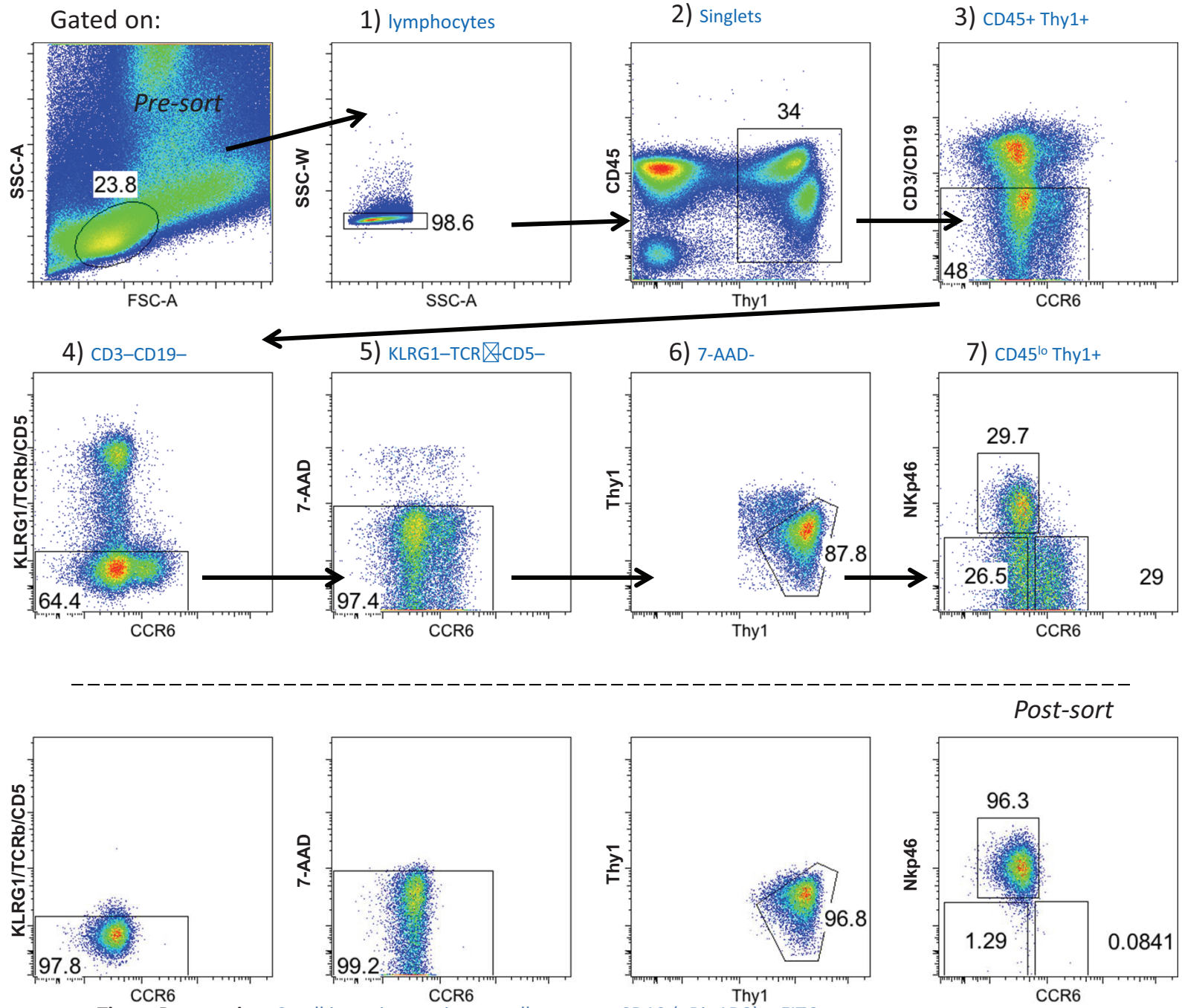
Michelle Robinette
Colonna Lab
Washington University in St. Louis

ILC3.NKp46+.SI: Small intestine NKp46+ ILC3



Tissue Preparation: Small intestine cut into small pieces, IEL removed by washing with EDTA, dissociated with Collagenase 4, lymphocyte isolation with Percoll gradient.

Time from Mouse to Trizol: 09:15am-03:00pm

Antibody (Clone) - Fluorochrome:

CD45 (30-F11) – APC-Cy7
CD3e (145-2C11) – FITC
CD19 (eBio1D3) – FITC
Thy1.2 (53-2.1) – APC
TCRb (H57-597) – PE
CD5 (53-7.3) – PE
KLRG1 (2F1) – PE
CCR6 (140706) – BV421
NKp46 (CS96) – biotin – SA-PeCy7

ILC3: 96.8%
NKp46+: 96.3%
purity

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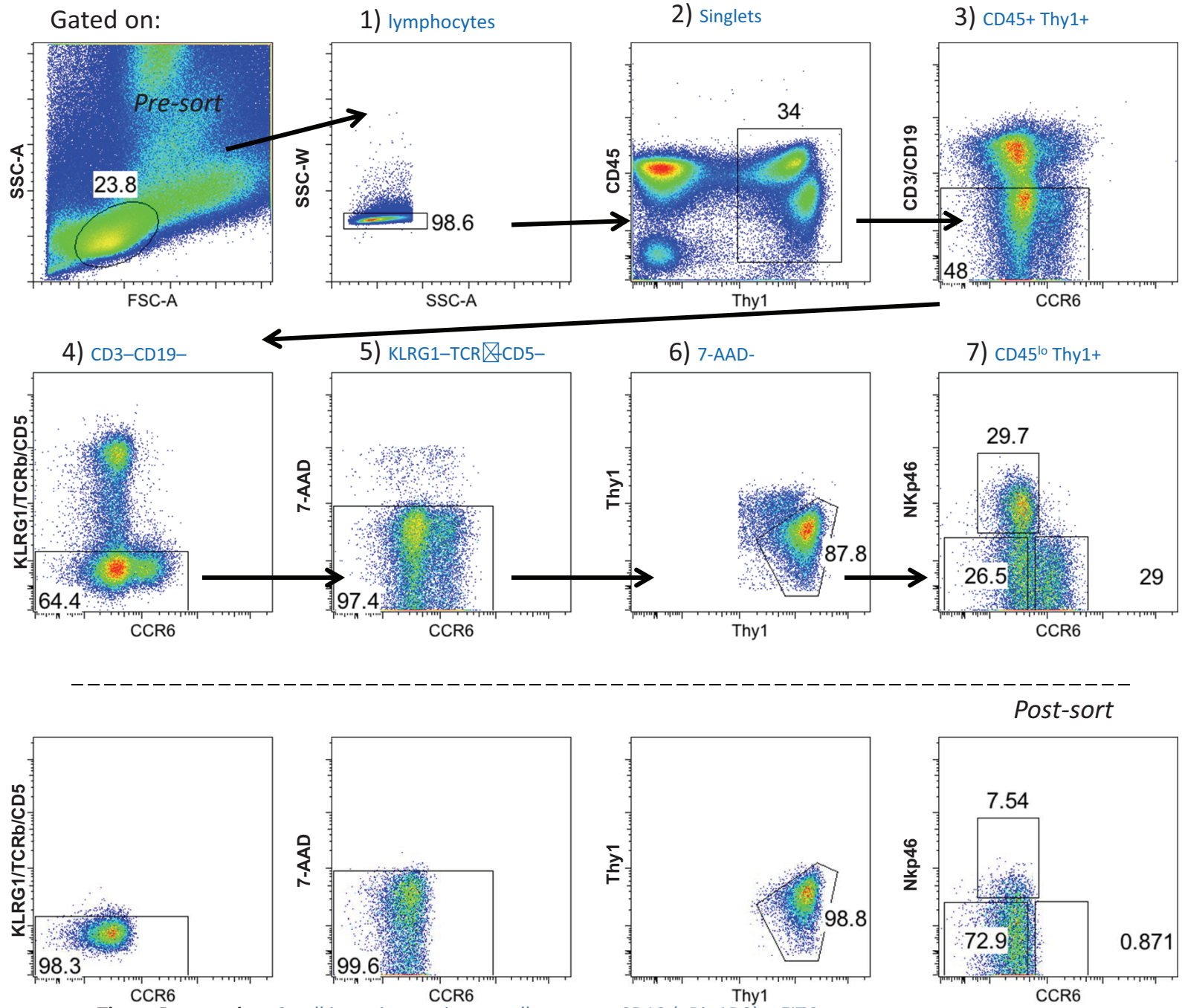
Michelle Robinette
Colonna Lab
Washington University in St. Louis

ILC3.NKp46-CCR6-.SI: Small intestine NKp46- CCR6- ILC3



Tissue Preparation: Small intestine cut into small pieces, IEL removed by washing with EDTA, dissociated with Collagenase 4, lymphocyte isolation with Percoll gradient.

Time from Mouse to Trizol: 09:15am-02:45pm

Antibody (Clone) - Fluorochrome:

CD45 (30-F11) – APC-Cy7
CD3e (145-2C11) – FITC
CD19 (eBio1D3) – FITC
Thy1.2 (53-2.1) – APC
TCRb (H57-597) – PE
CD5 (53-7.3) – PE
KLRG1 (2F1) – PE
CCR6 (140706) – BV421
NKp46 (CS96) – biotin – SA-PeCy7

ILC3: 98.8%
DN: 72.9%
purity

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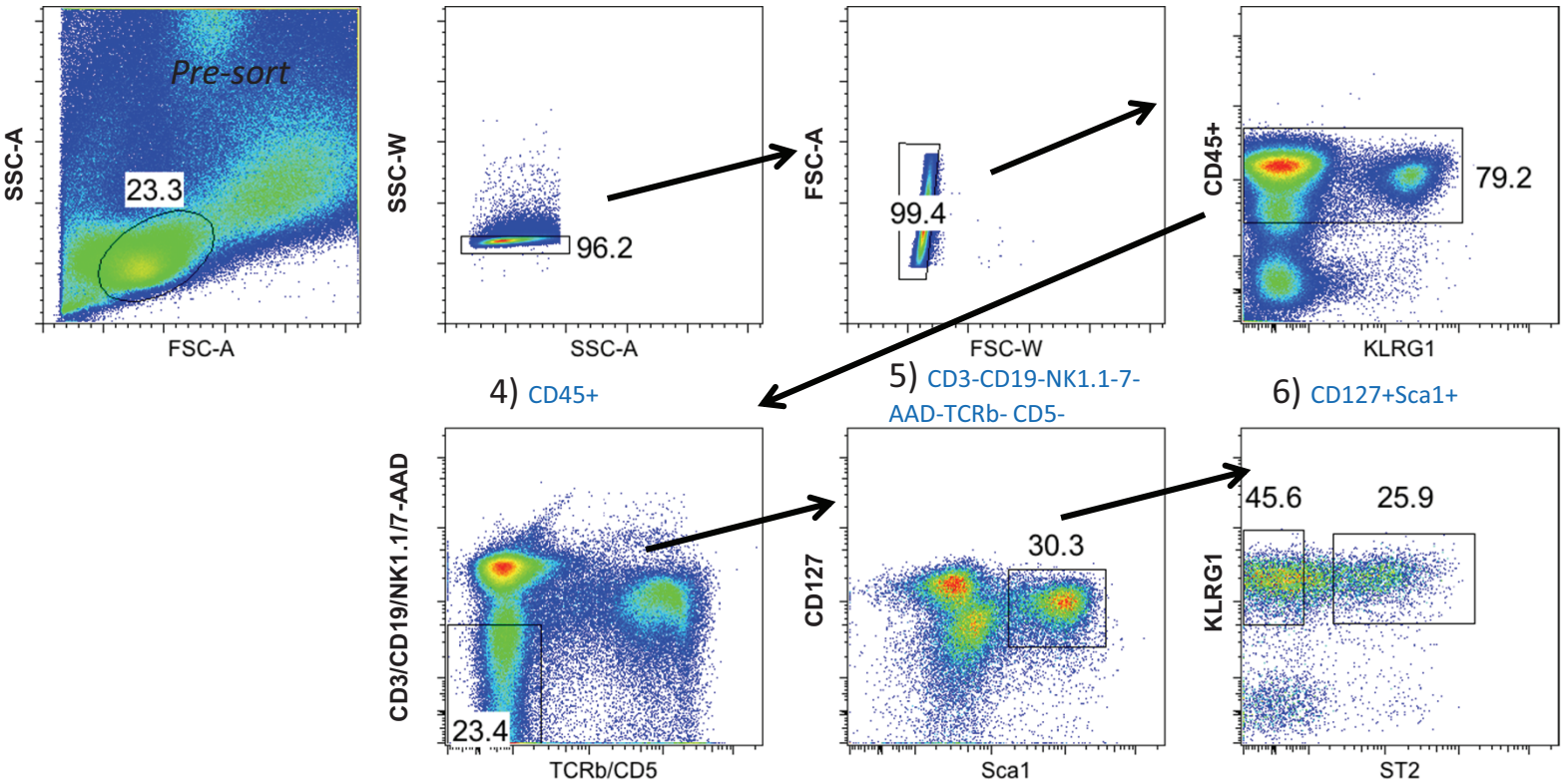
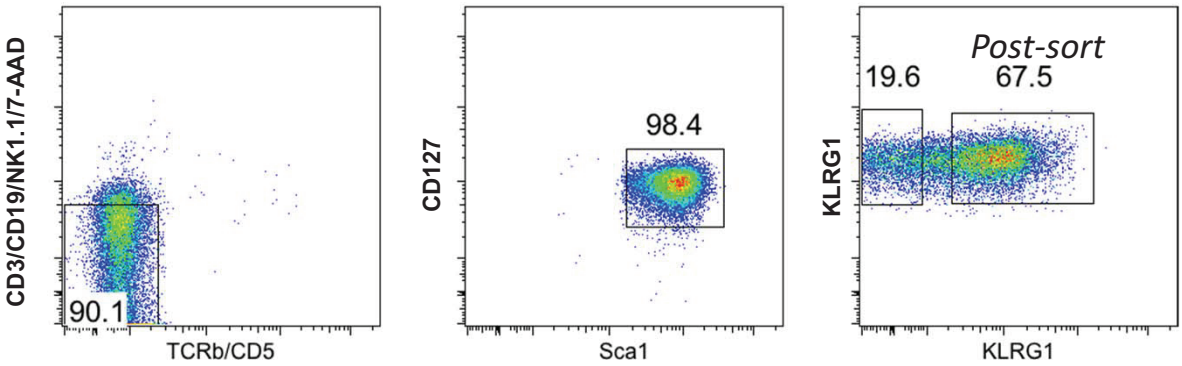
Michelle Robinette
Colonna Lab
Washington University in St. Louis

ILC2.SI: Small intestine ILC2

Gated on:





Tissue Preparation: Small intestine cut into small pieces, IEL removed by washing with EDTA, dissociated with Collagenase 4, lymphocyte isolation with Percoll gradient.

Time from Mouse to Trizol: 08:45am-01:45pm

Antibody (Clone) - Fluorochrome:

CD45 (30-F11) – APC-Cy7
CD3e (145-2C11) – PerCP-Cy5.5
CD19 (eBio1D3) – PerCP-Cy5.5
NK1.1 (PK136) – PerCP-Cy5.5
TCR (H57-597) – PE
CD5 (53-7.3) – PE
Sca1 (D7) – PacBlue
CD127 (A7R34) – FITC
KLRG1 (2F1) – APC
ST2 (RMST-2) – biotin – SA-PeCy7

ILC2: 98.4%
$ST2^+$:67.5%
purity

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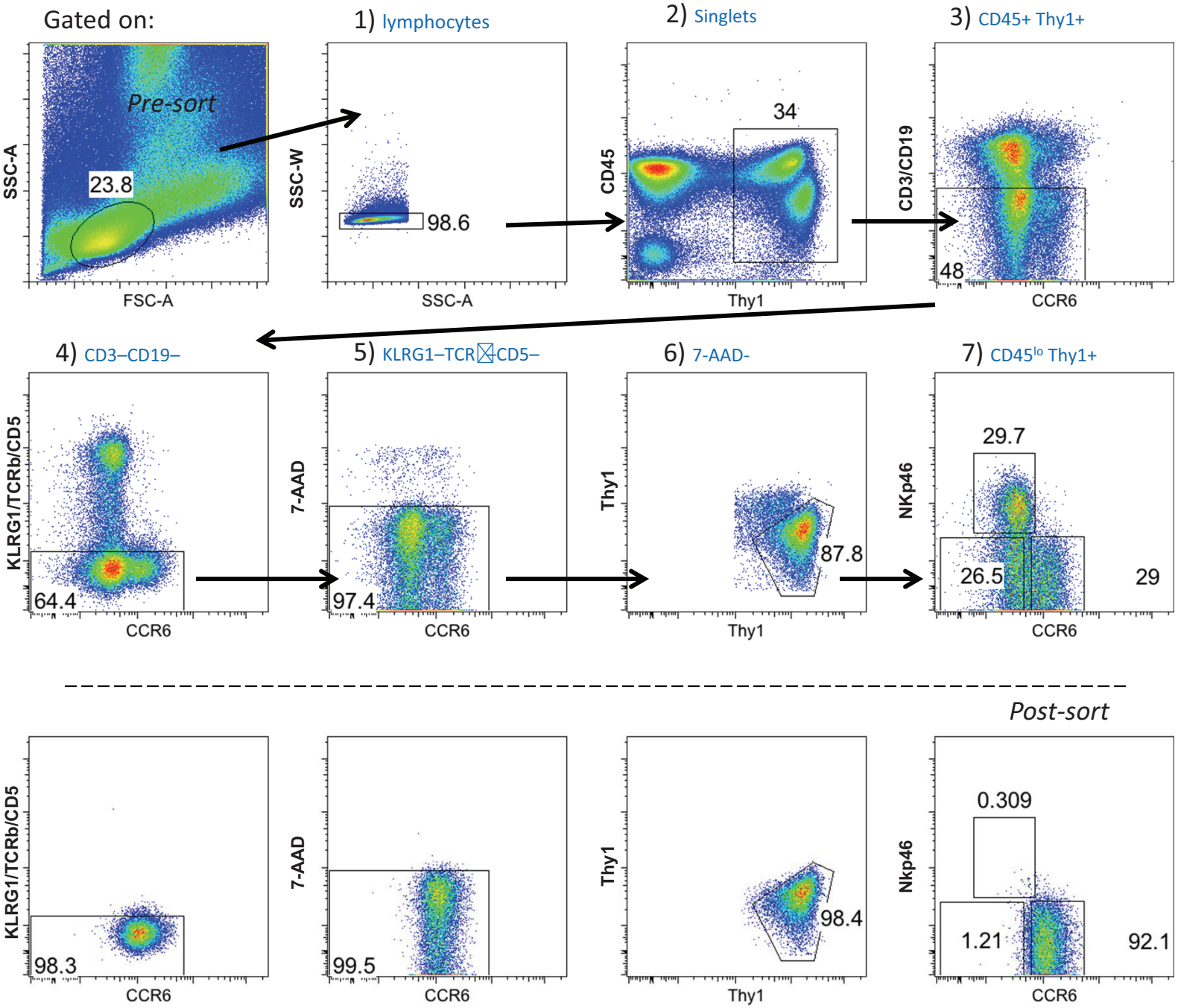
Michelle Robinette
Colonna Lab
Washington University in St. Louis

ILC3.CCR6+.SI: Small intestine CCR6+ ILC3



Tissue Preparation: Small intestine cut into small pieces, IEL removed by washing with EDTA, dissociated with Collagenase 4, lymphocyte isolation with Percoll gradient.

Time from Mouse to Trizol: 09:15am-02:30pm

Antibody (Clone) - Fluorochrome:

CD45 (30-F11) – APC-Cy7
CD3e (145-2C11) – FITC
CD19 (eBio1D3) – FITC
Thy1.2 (53-2.1) – APC
TCR⊠(H57-597) – PE
CD5 (53-7.3) – PE
KLRG1 (2F1) – PE
CCR6 (140706) – BV421
NKp46 (CS96) – biotin – SA-PeCy7

ILC3: 98.4%
CCR6+: 92.1%
purity

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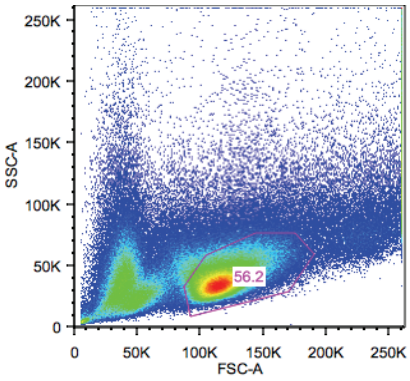
University of California, San Diego

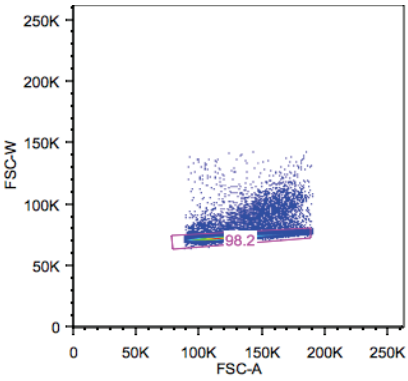
T.8.MP.LCMV.d7.Sp: Splenic CD8 T-cell memory precursors, day 7 post LCMV

Pre-sort

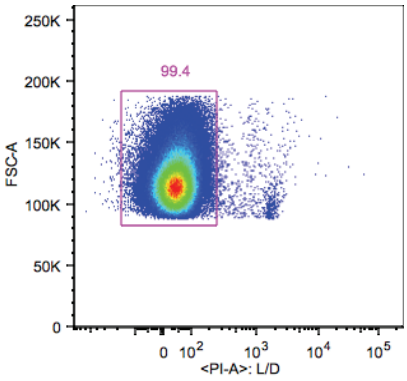
1) Lymphocytes



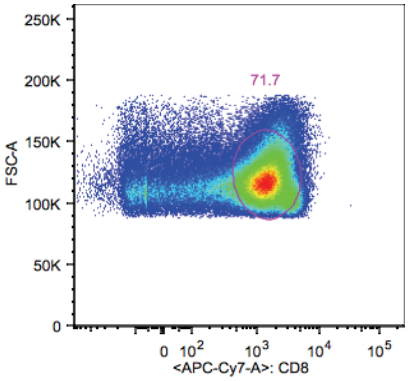
2) Singlet Cells



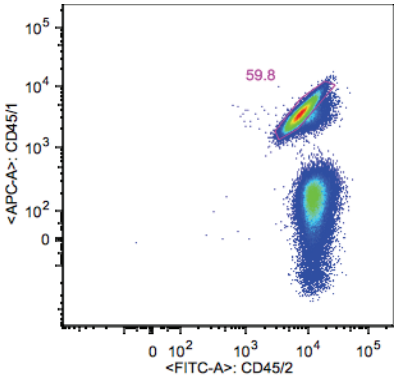
3) PI-



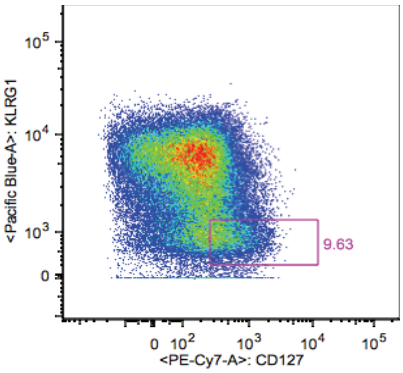
4) CD8+ T cells



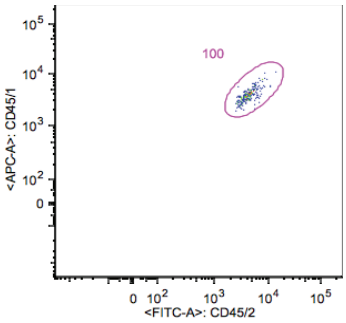
5) Donor cells

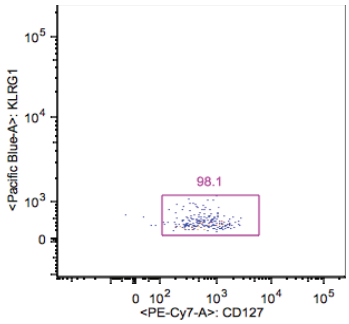


6) KLRG1-CD127+



Post-sort





Tissue Preparation:
Glass slide Teasing

Time from Mouse to the 2nd sorting:
9:00am-1:00pm

Antibody (Clone) - Fluorochrome:
CCD8a(53-6.7) -- APC-Cy7
CD45.2(104) -- FITC
CD45.1(A20) -- APC
CD127(A7R34) -- PE-Cy7
KLRG1(2F1) -- Pacific Blue

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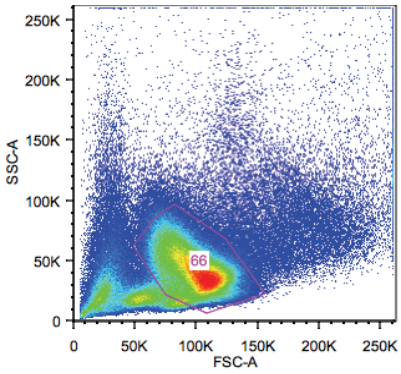
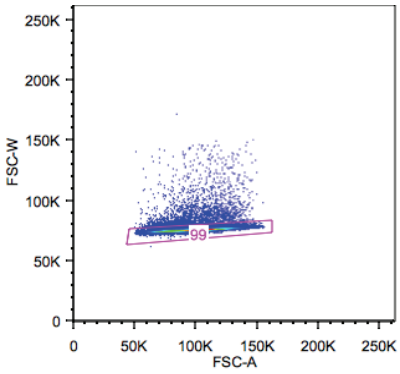
T.8.Tcm.LCMV.d180.Sp: Splenic central memory CD8 T cells, day 180 post LCMV

Pre-sort

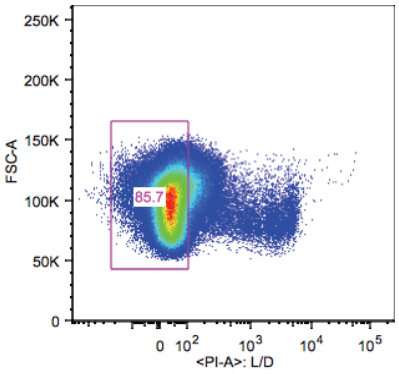
1) Lymphocytes



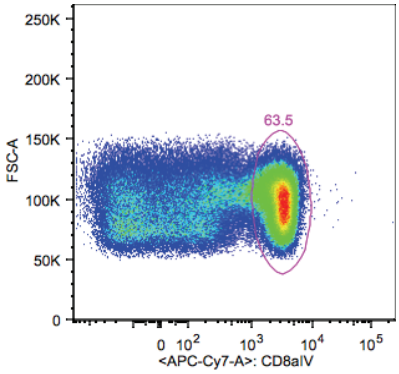
2) Singlet Cells



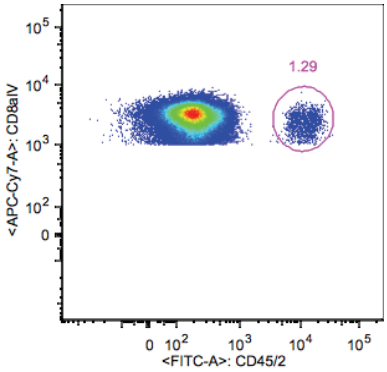
3) PI-



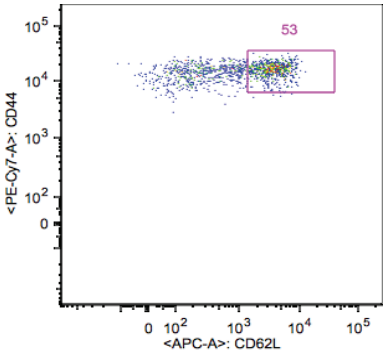
4) CD8+ T cells



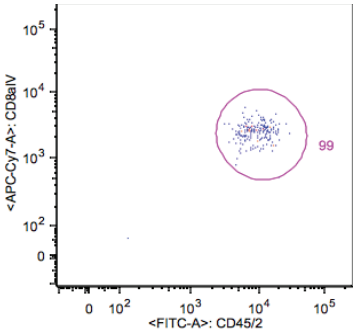
5) Donor cells

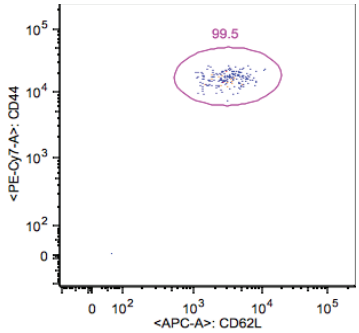


6) CD44+CD62L+



Post-sort





Tissue Preparation:
Glass slide Teasing

Time from Mouse to the 2nd sorting:
9:00am-1:00pm

Antibody (Clone) - Fluorochrome:
CCD8a(53-6.7) -- APC-Cy7
CD45.2(104) -- FITC
CD44(IM7) -- PE-Cy7
CD62L(MEL-14) -- APC

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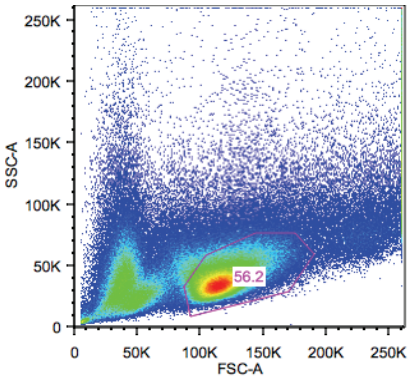
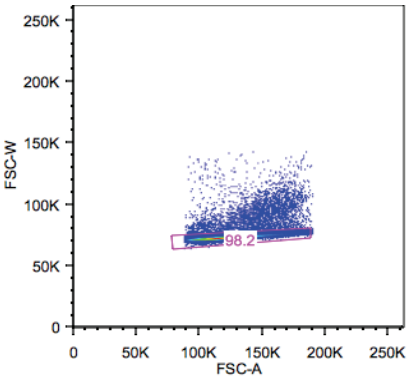
T.8.TE.LCMV.d7.Sp: Splenic termnal effector CD8 T cells, day 8 post LCMV

Pre-sort

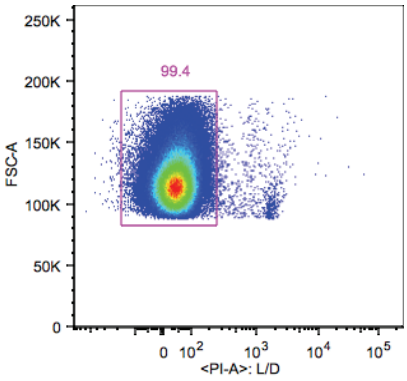
1) Lymphocytes



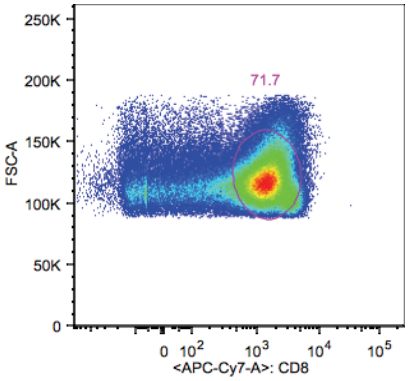
2) Singlet Cells



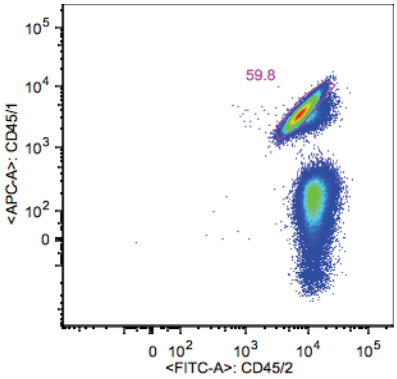
3) PI-



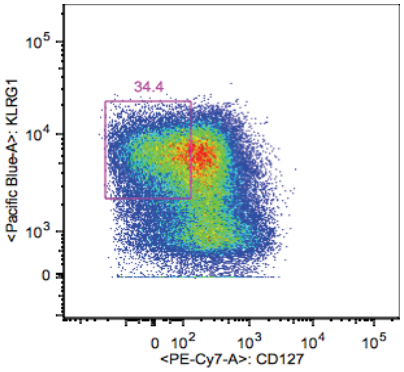
4) CD8+ T cells



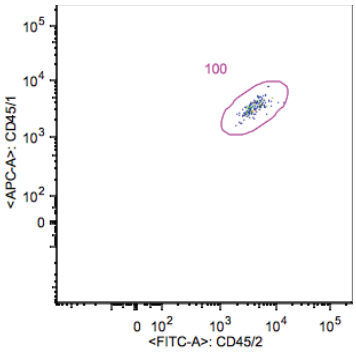
5) Donor cells

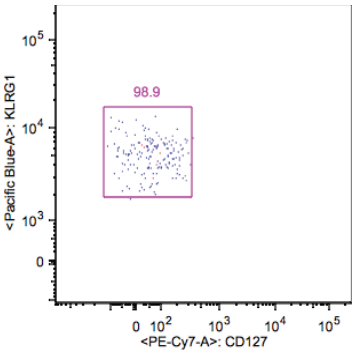


6) KLRG1+CD127-



Post-sort





Tissue Preparation:
Glass slide Teasing

Time from Mouse to the 2nd sorting:
9:00am-1:00pm

Antibody (Clone) - Fluorochrome:
CCD8a(53-6.7) -- APC-Cy7
CD45.2(104) -- FITC
CD45.1(A20) -- APC
CD127(A7R34) -- PE-Cy7
KLRG1(2F1) -- Pacific Blue

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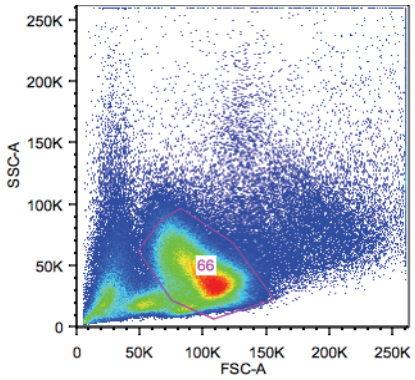
University of California, San Diego

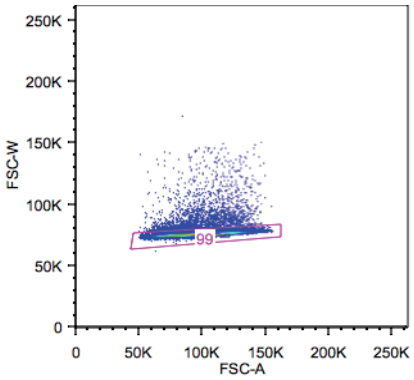
T.8.Tem.LCMV.d180.Sp: Splenic effector memory CD8 T cells, day 180 post LCMV

Pre-sort

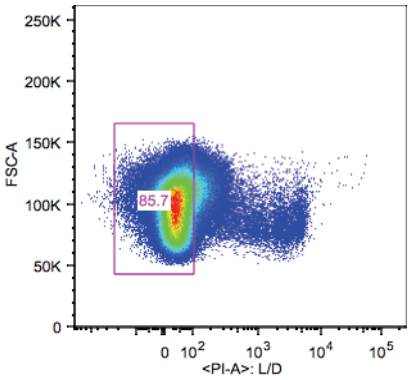
1) Lymphocytes



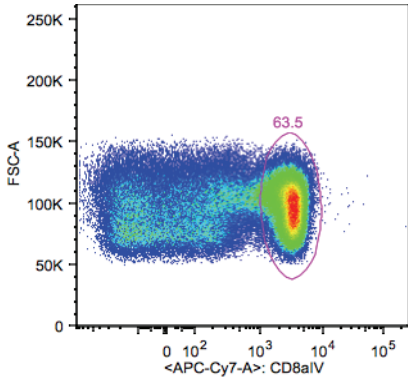
2) Singlet Cells



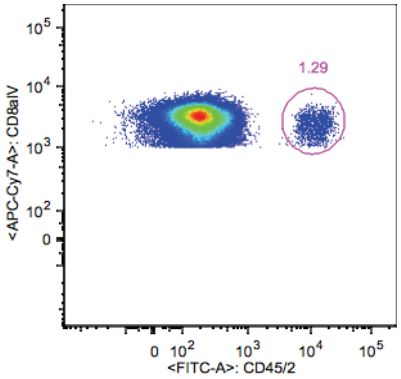
3) PI-



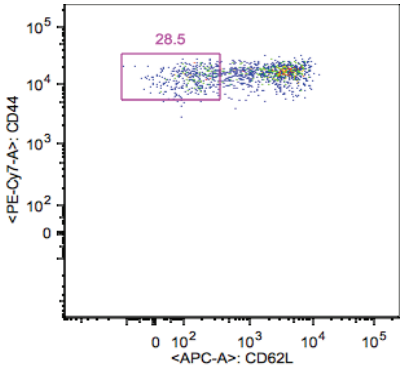
4) CD8+ T cells



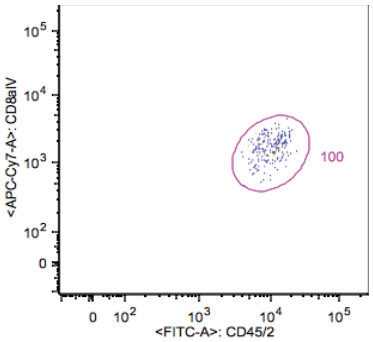
5) Donor cells

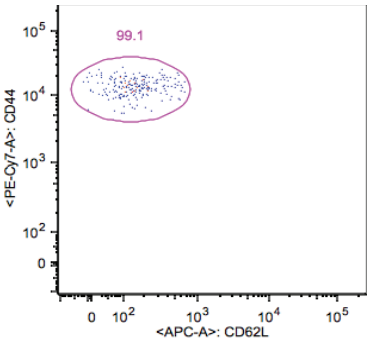


6) CD44+CD62L



Post-sort





Tissue Preparation:
Glass slide Teasing

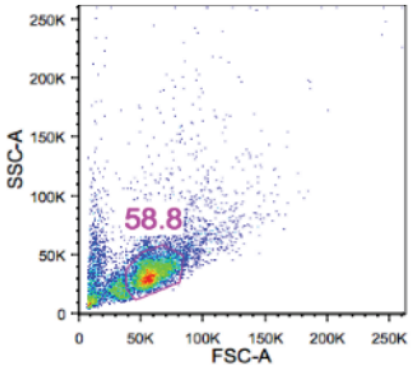
Time from Mouse to the 2nd sorting:
9:00am-1:00pm

Antibody (Clone) - Fluorochrome:
CCD8a(53-6.7) -- APC-Cy7
CD45.2(104) -- FITC
CD44(IM7) -- PE-Cy7
CD62L(MEL-14) -- APC

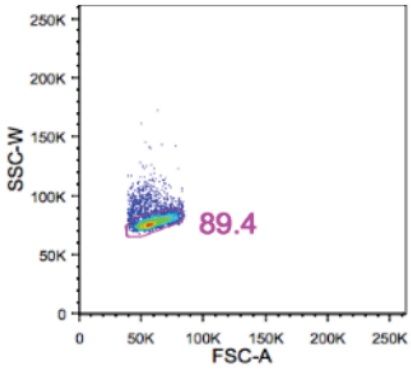
T.8.TN.P14.Sp: Splenic naive CD8 T cells from P14 TCR Tg mice

Pre-sort

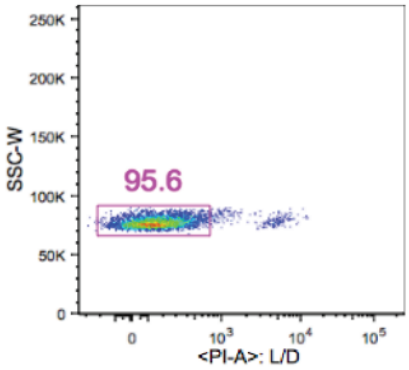
1) Lymphocytes



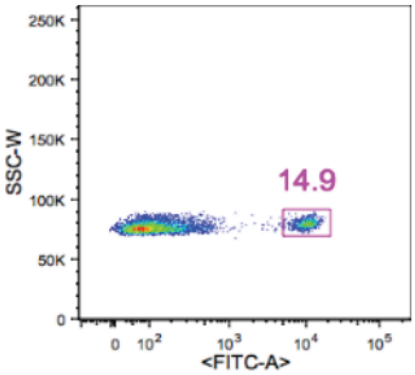
2) Singlet Cells



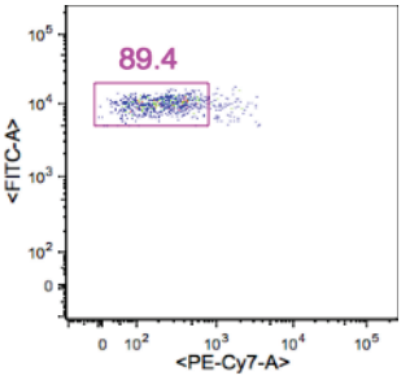
3) PI-



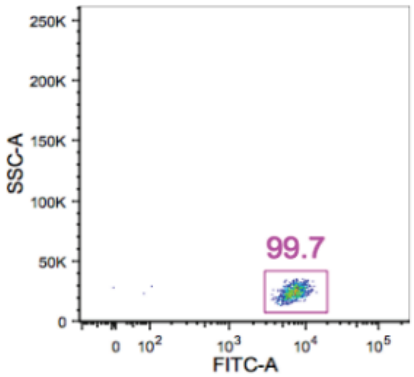
4) CD8+ T cells

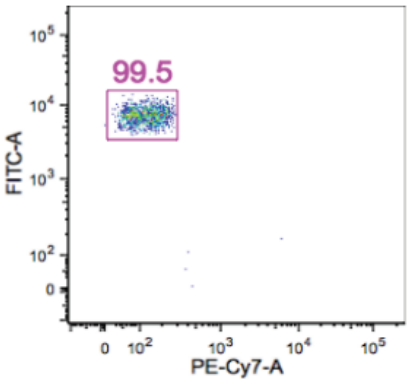


5) CD44-CD8+



Post-sort





Tissue Preparation:
Glass slide Teasing

Time from Mouse to the 2nd sorting:
9:00am-1:00pm

Antibody (Clone) - Fluorochrome:
CD8a(53-6.7) -- FITC
CD44(IM7) -- PE-Cy7

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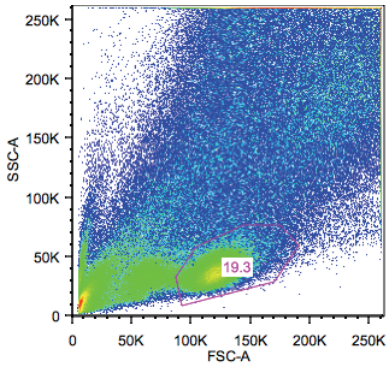
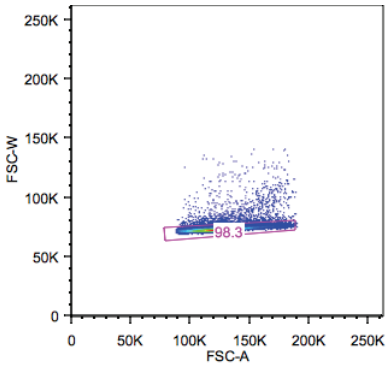
T.8.IEL.LCMV.d7.SI: Small intestine intraepithelial lymphocytes, day7 post LCMV

Pre-sort

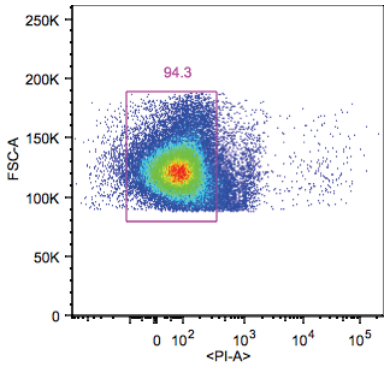
1) Lymphocytes



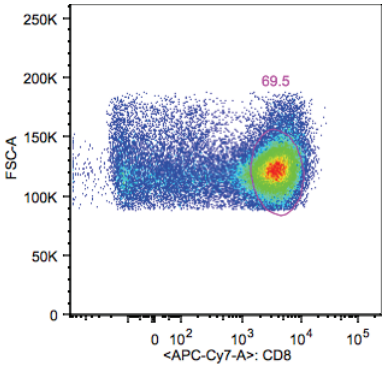
2) Singlet Cells



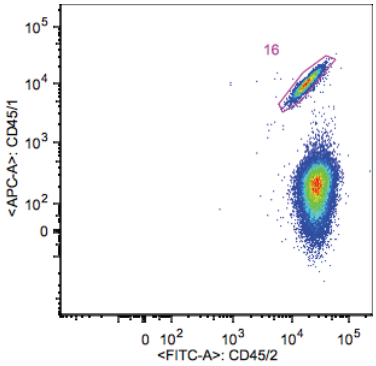
3) PI-



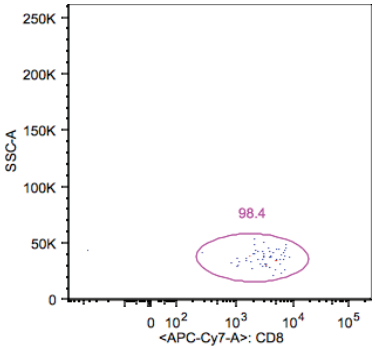
4) CD8+ T cells

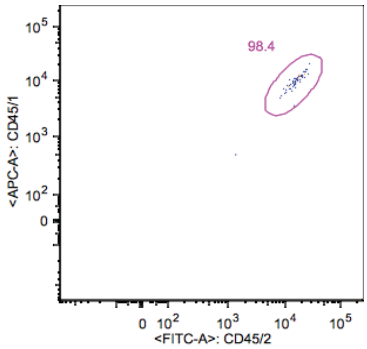


5) Donor cells



Post-sort





Tissue Preparation:
DTE treated then isolated by Percoll gradient

Time from Mouse to the 2nd sorting:
9:00am-2:00pm

Antibody (Clone) - Fluorochrome:
CCD8a(53-6.7) -- APC-Cy7
CD45.2(104) -- FITC
CD45.1(A20) -- APC

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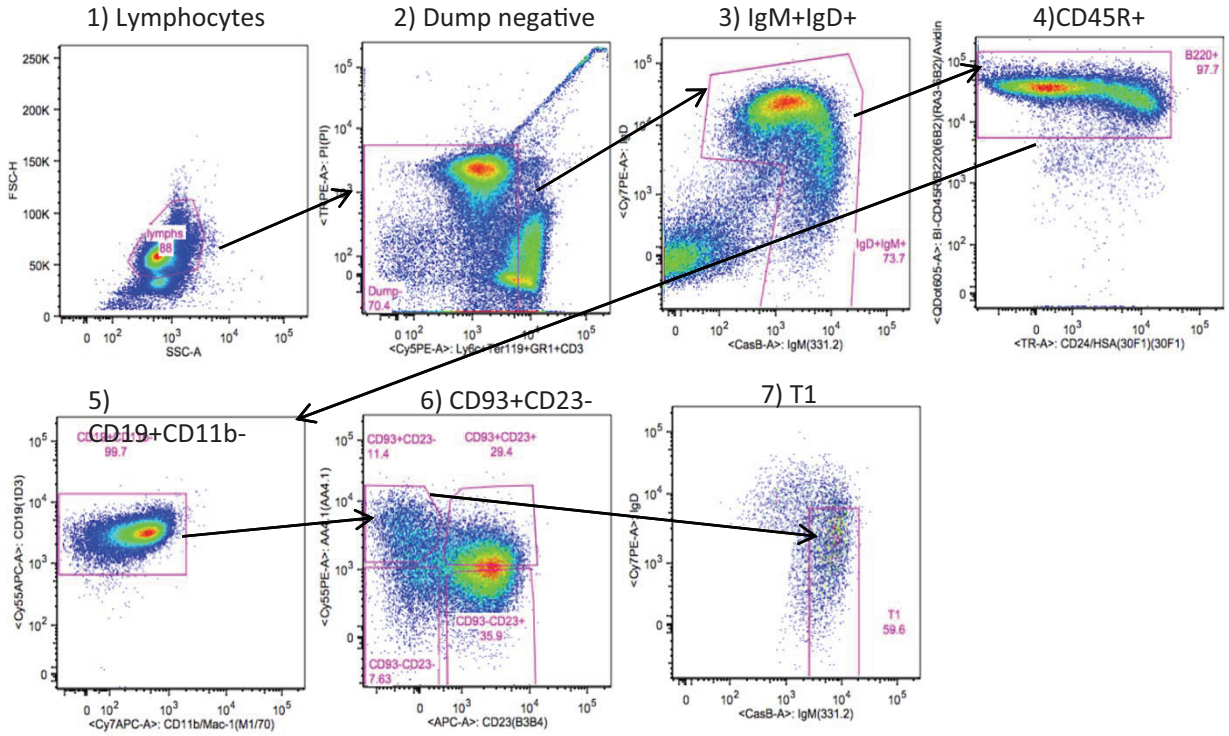
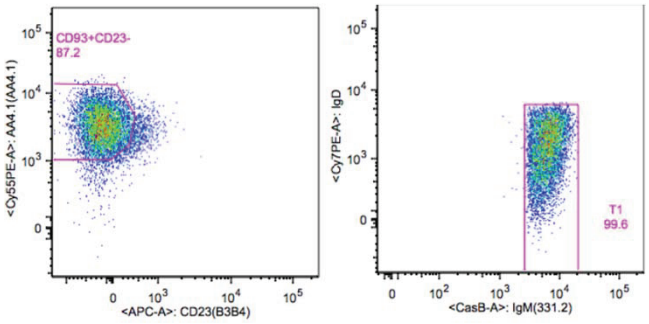
FOX CHASE CANCER CENTER

B.T1.Sp: Splenic T1 B Cells

Pre-sort



Post-sort



Tissue Preparation: Grind between slides, ACK lysis of RBCs
Time from Mouse to Trizol: 8:30AM-11:30AM

Antibody (Clone) - Fluorochrome:
CD43(S7)-FITC; CD21/35-PE; CD3(500A-A2),Ter119, Ly6c(AL21), GR1(8C5)-Cy5PE; CD93(AA4.1)-Cy55PE; IgD(JA12.5)-Cy7PE; CD23(B3B4)-APC; CD19(1D3)-Cy55APC; CD11b(Mac1)-Cy7APC; IgM(331.12)-Cascade Blue; CD45R(6B2)-biotin/Q Dot 605 Avidin

T1 cells 99.6% purity

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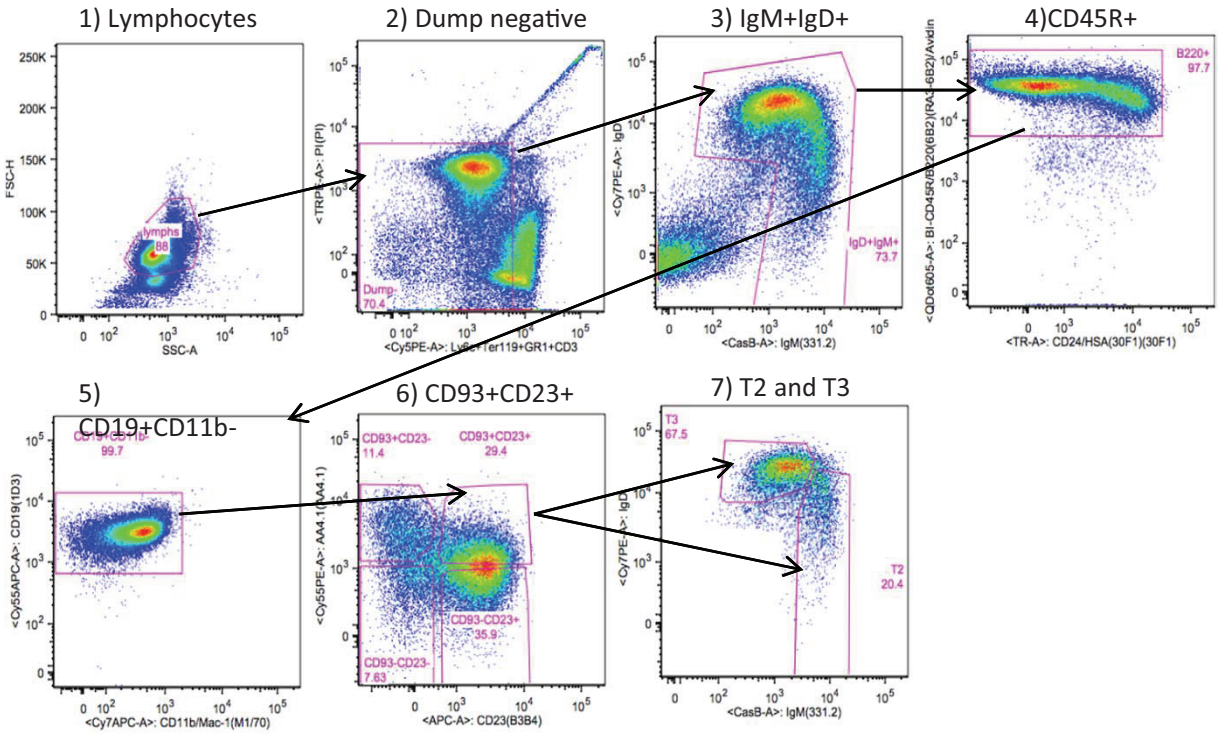
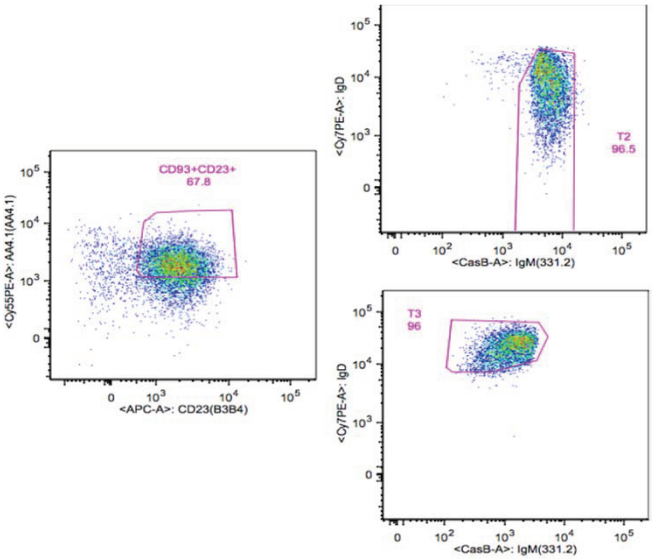
B.T2.Sp: Splenic T2 B Cells

Pre-sort



Post-sort



Tissue Preparation: Grind between slides, ACK lysis of RBCs
Time from Mouse to Trizol: 8:30AM-11:30AM

Antibody (Clone) - Fluorochrome:
CD43(S7)-FITC; CD21/35-PE; CD3(500A-A2),Ter119, Ly6c(AL21), GR1(8C5)-Cy5PE; CD93(AA4.1)-Cy55PE; IgD(JA12.5)-Cy7PE; CD23(B3B4)-APC; CD19(1D3)-Cy55APC; CD11b(Mac1)-Cy7APC; IgM(331.12)-Cascade Blue; CD45R(6B2)-biotin/Q Dot 605 Avidin

T2 cells 96.5% purity, T3 cells 96% purity

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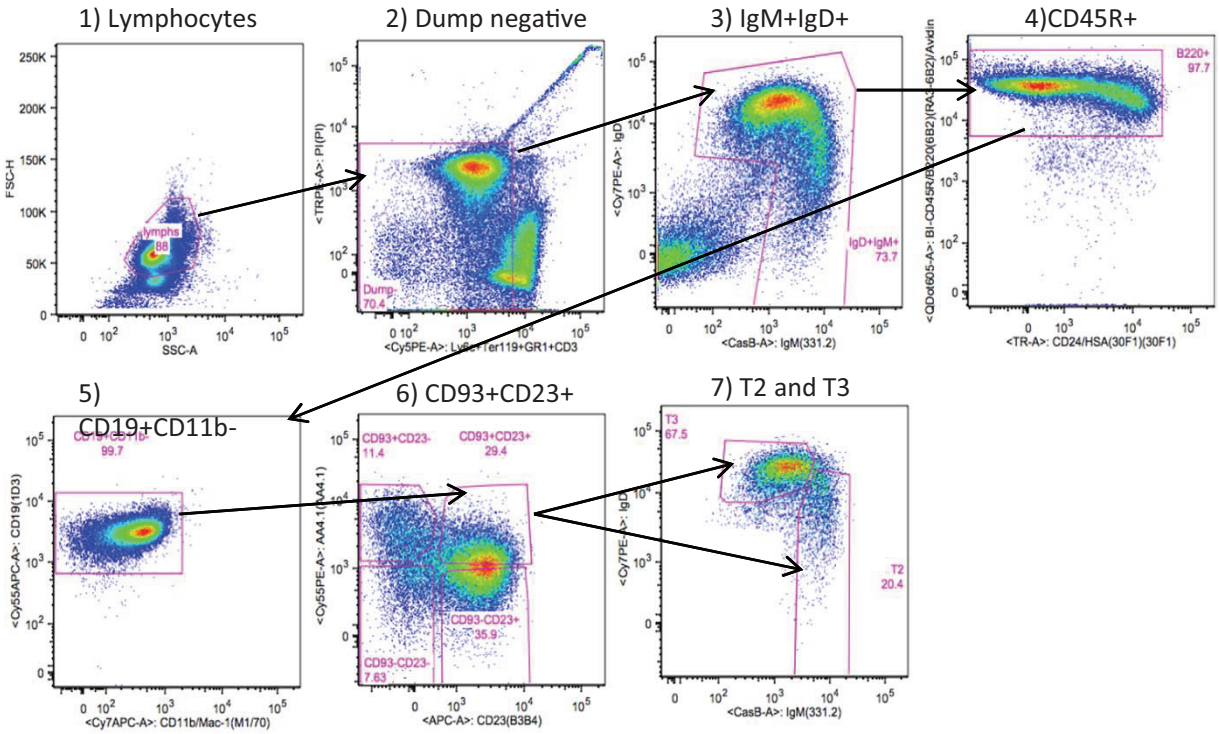
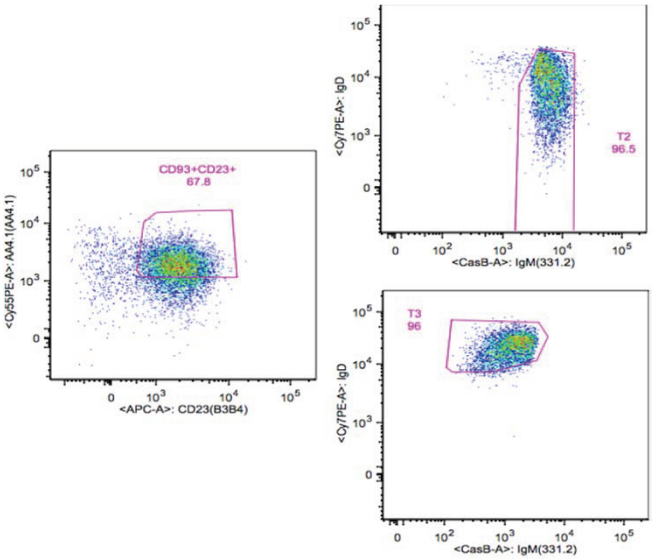
FOX CHASE CANCER CENTER

B.T3.Sp: Splenic T3 B Cells

Pre-sort



Post-sort



Tissue Preparation: Grind between slides, ACK lysis of RBCs
Time from Mouse to Trizol: 8:30AM-11:30AM

Antibody (Clone) - Fluorochrome:
CD43(S7)-FITC; CD21/35-PE; CD3(500A-A2),Ter119, Ly6c(AL21), GR1(8C5)-Cy5PE; CD93(AA4.1)-Cy55PE; IgD(JA12.5)-Cy7PE; CD23(B3B4)-APC; CD19(1D3)-Cy55APC; CD11b(Mac1)-Cy7APC; IgM(331.12)-Cascade Blue; CD45R(6B2)-biotin/Q Dot 605 Avidin

T2 cells 96.5% purity, T3 cells 96% purity

Immunological Genome Project

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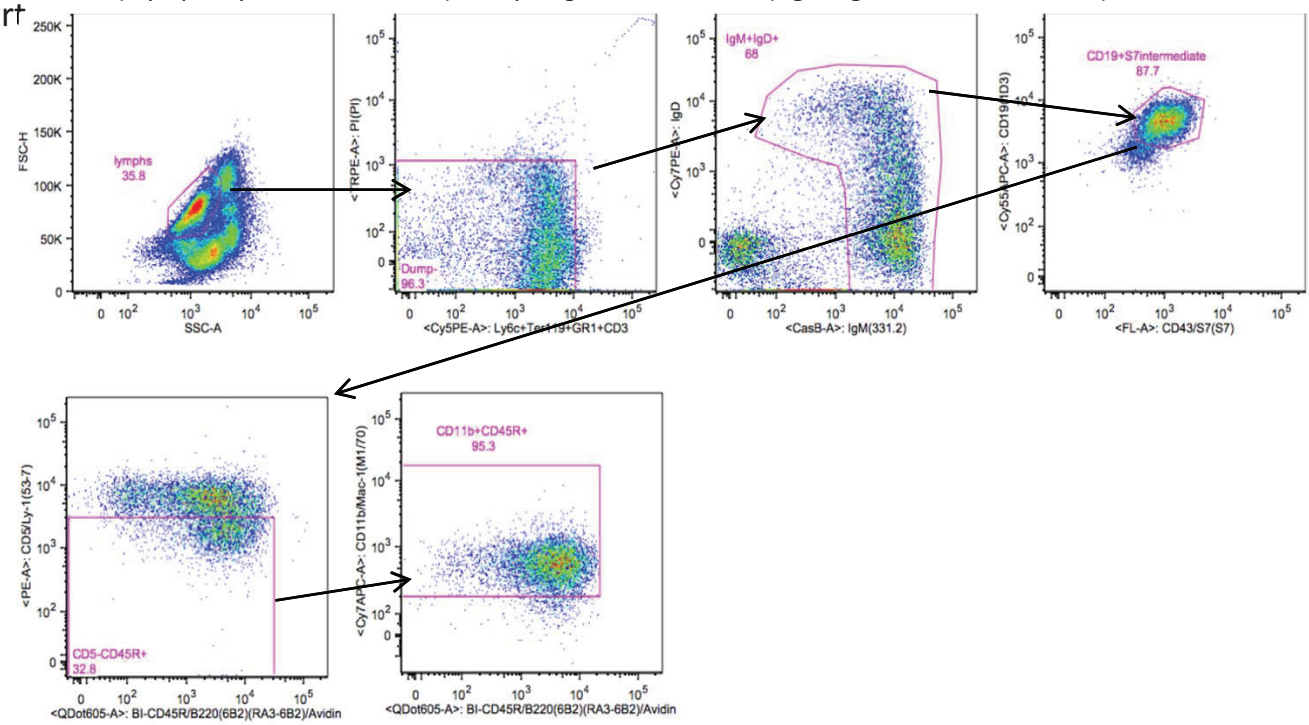
FOX CHASE CANCER CENTER

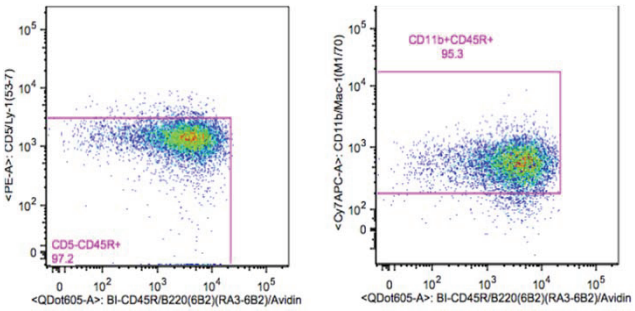
B1b.PC: Peritoneal Cavity B1b B Cells

Pre-sort

1) Lymphocytes 2) Dump negative 3) IgM+IgD+ 4) CD19+CD43+



Post-sort



Tissue Preparation: Peritoneal cavity washout

Time from Mouse to Trizol: 8:30AM-10:30AM

Antibody (Clone) - Fluorochrome:
CD43(S7)-FITC; CD5(53-7)-PE; CD3(500A-A2),Ter119, Ly6c(AL21), GR1(8C5)-Cy5PE; IgD(JA12.5)-Cy7PE; CD23(B3B4)-APC; CD19(1D3)-Cy55APC; CD11b(Mac1)-Cy7APC; IgM(331.12)-Cascade Blue; CD45R(6B2)-biotin/ Q Dot 605 Avidin

B1b cells 93.3% purity

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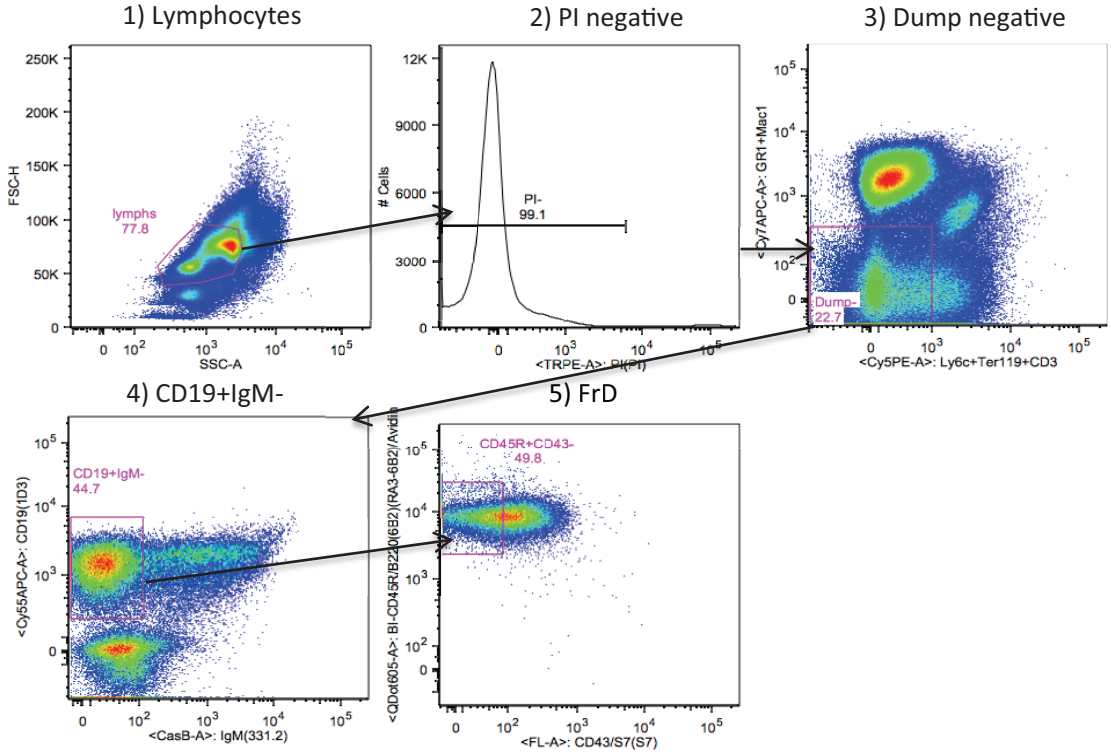
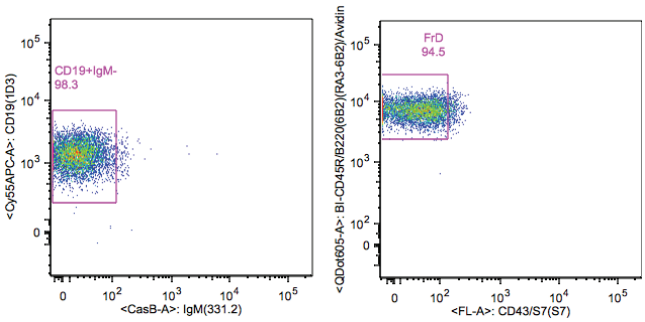
preB.FrD.BM: Bone marrow pre-B cells (Fraction D)

Pre-sort



Post-sort



Tissue Preparation: Harvest cells from BM using 1ml syringe, ACK lysis of RBCs

Time from Mouse to Trizol: 8:30AM-1:30PM

Antibody (Clone) - Fluorochrome:

CD43(S7)-FITC; CD127(SB/199)-PE; CD3(500A-A2),Ter119, Ly6c(AL21); CD93(AA4.1)-Cy55PE; CD24(30F1)-Alexa594; CD117(2B8)-APC; CD19(1D3)-Cy55APC; GR1(8c5), CD11b(Mac1)-Cy7APC; IgM(331.12)-Cascade Blue; CD45R(6B2)-biotin/Q Dot 605 Avidin

FrD cells 94.5% purity

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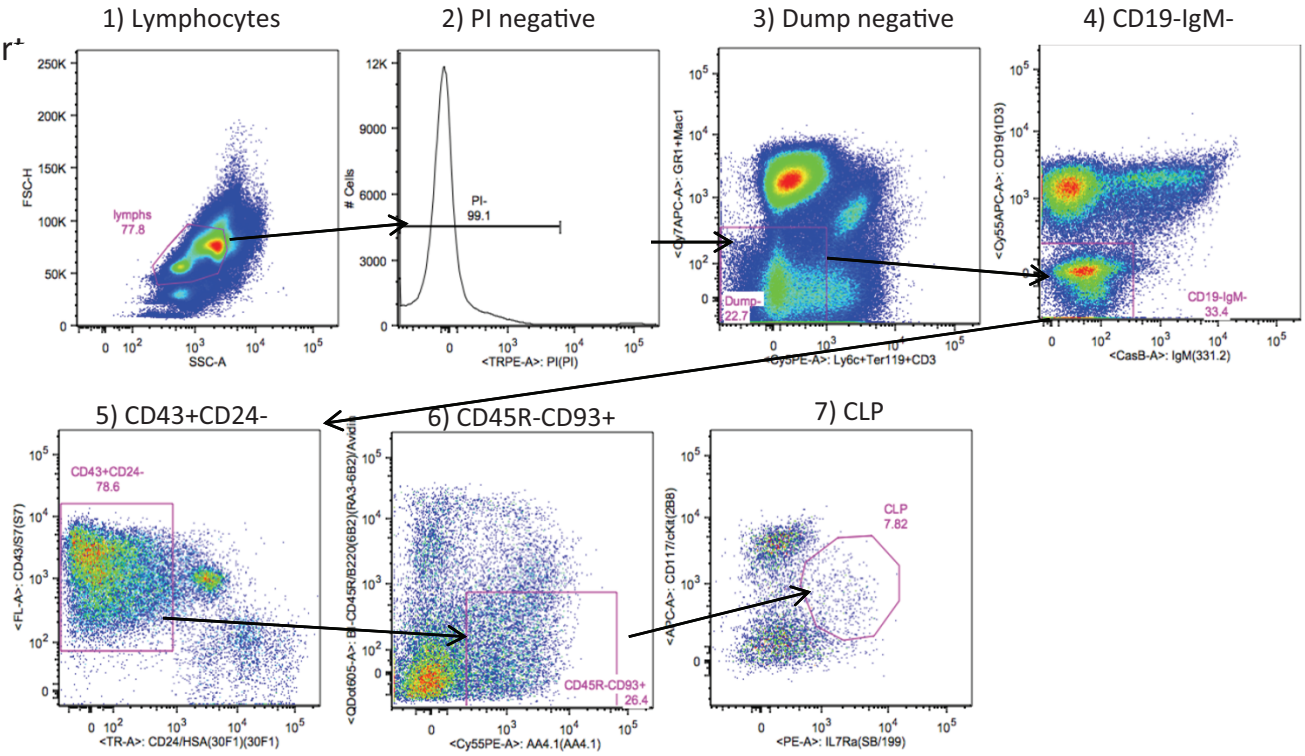
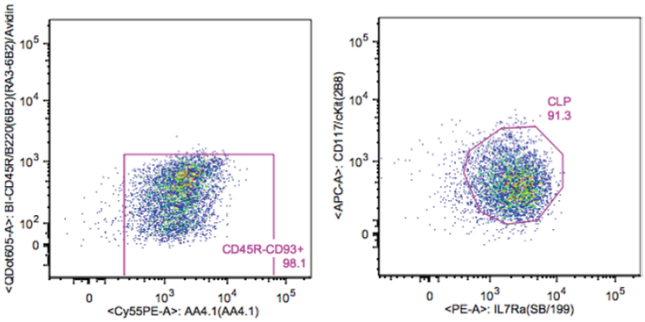
proB.CLP.BM: Bone marrow pro-B cells (common lymphoid progenitors)

Pre-sort



Post-sort



Tissue Preparation: Harvest cells from BM using 1ml syringe, ACK lysis of RBCs

Time from Mouse to Trizol: 8:30AM-1:30PM

Antibody (Clone) - Fluorochrome:

CD43(S7)-FITC; CD127(SB/199)-PE; CD3(500A-A2),Ter119, Ly6c(AL21); CD93(AA4.1)-Cy55PE; CD24(30F1)-Alexa594; CD117(2B8)-APC; CD19(1D3)-Cy55APC; GR1(8c5), CD11b(Mac1)-Cy7APC; IgM(331.12)-Cascade Blue; CD45R(6B2)-biotin/Q Dot 605 Avidin

CLP cells 91.3% purity

Immunological Genome Project

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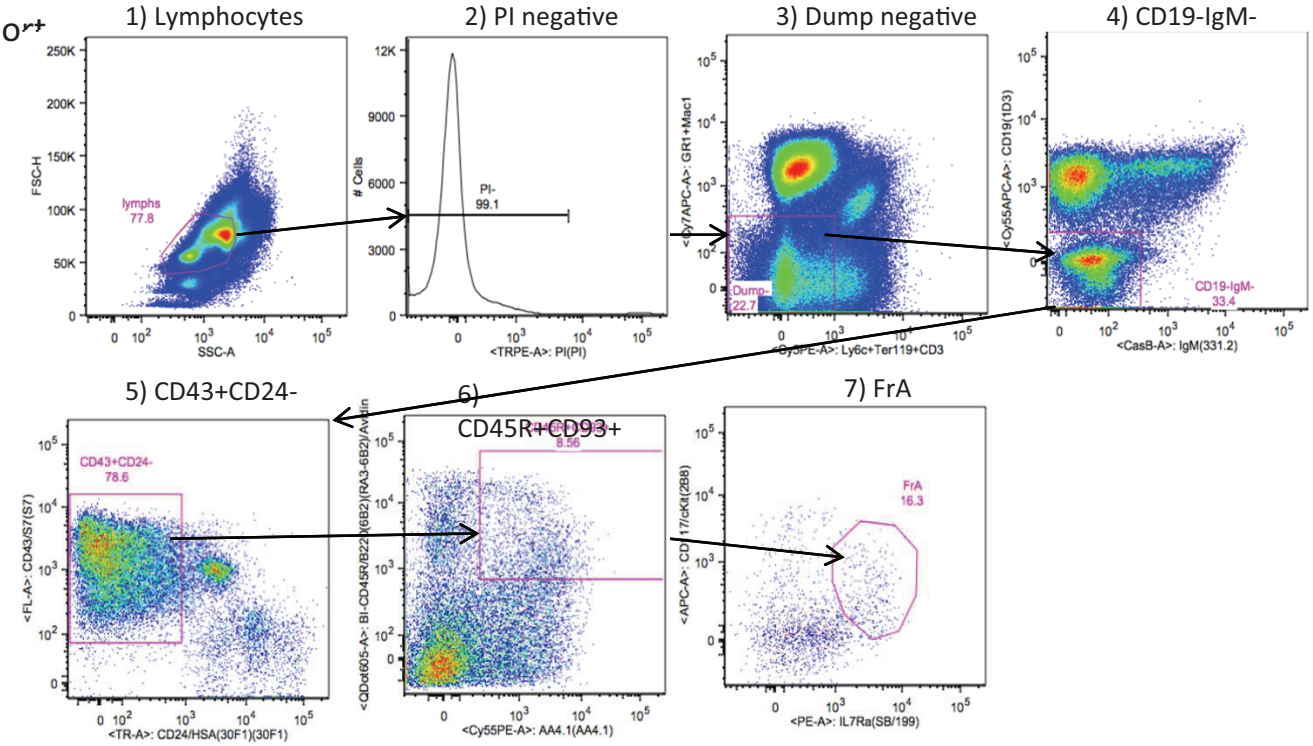
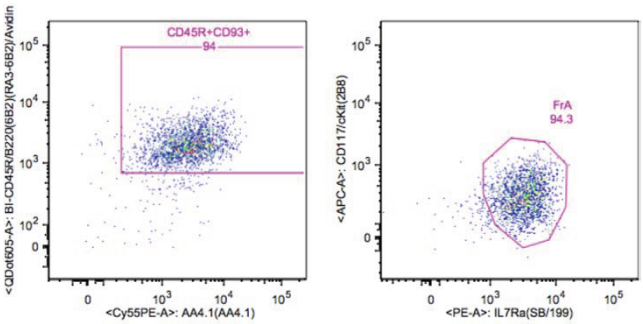
FOX CHASE CANCER CENTER

proB.FrA.BM: Bone marrow pro-B cells (Fraction A)

Pre-sort



Post-sort



Tissue Preparation: Harvest cells from BM using 1ml syringe, ACK lysis of RBCs

Time from Mouse to Trizol: 8:30AM-1:30PM

Antibody (Clone) - Fluorochrome:

CD43(S7)-FITC; CD127(SB/199)-PE; CD3(500A-A2),Ter119, Ly6c(AL21); CD93(AA4.1)-Cy55PE; CD24(30F1)-Alexa594; CD117(2B8)-APC; CD19(1D3)-Cy55APC; GR1(8c5), CD11b(Mac1)-Cy7APC; IgM(331.12)-Cascade Blue; CD45R(6B2)-biotin/Q Dot 605 Avidin

FrA cells 94.3% purity

Immunological Genome Project

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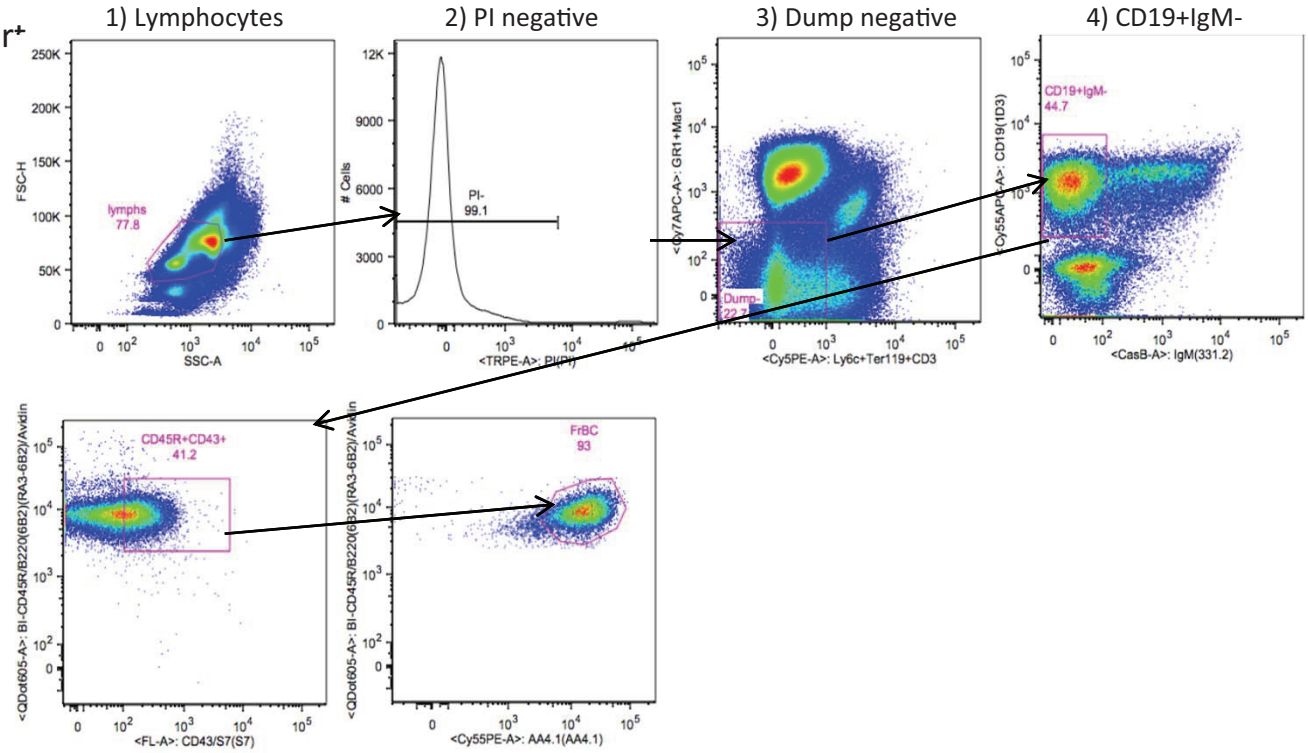
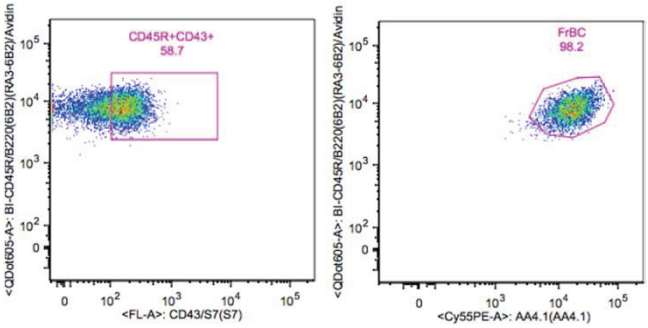
FOX CHASE CANCER CENTER

proB.FrBC.BM: Bone marrow pro-B cells (Fraction B/C)

Pre-sort



Post-sort



Tissue Preparation: Harvest cells from BM using 1ml syringe, ACK lysis of RBCs

Time from Mouse to Trizol: 8:30AM-1:30PM

Antibody (Clone) - Fluorochrome:

CD43(S7)-FITC; CD127(SB/199)-PE; CD3(500A-A2),Ter119, Ly6c(AL21); CD93(AA4.1)-Cy55PE; CD24(30F1)-Alexa594; CD117(2B8)-APC; CD19(1D3)-Cy55APC; GR1(8c5), CD11b(Mac1)-Cy7APC; IgM(331.12)-Cascade Blue; CD45R(6B2)-biotin/Q Dot 605 Avidin

FrB/C cells 98.2% purity

Immunological Genome Project

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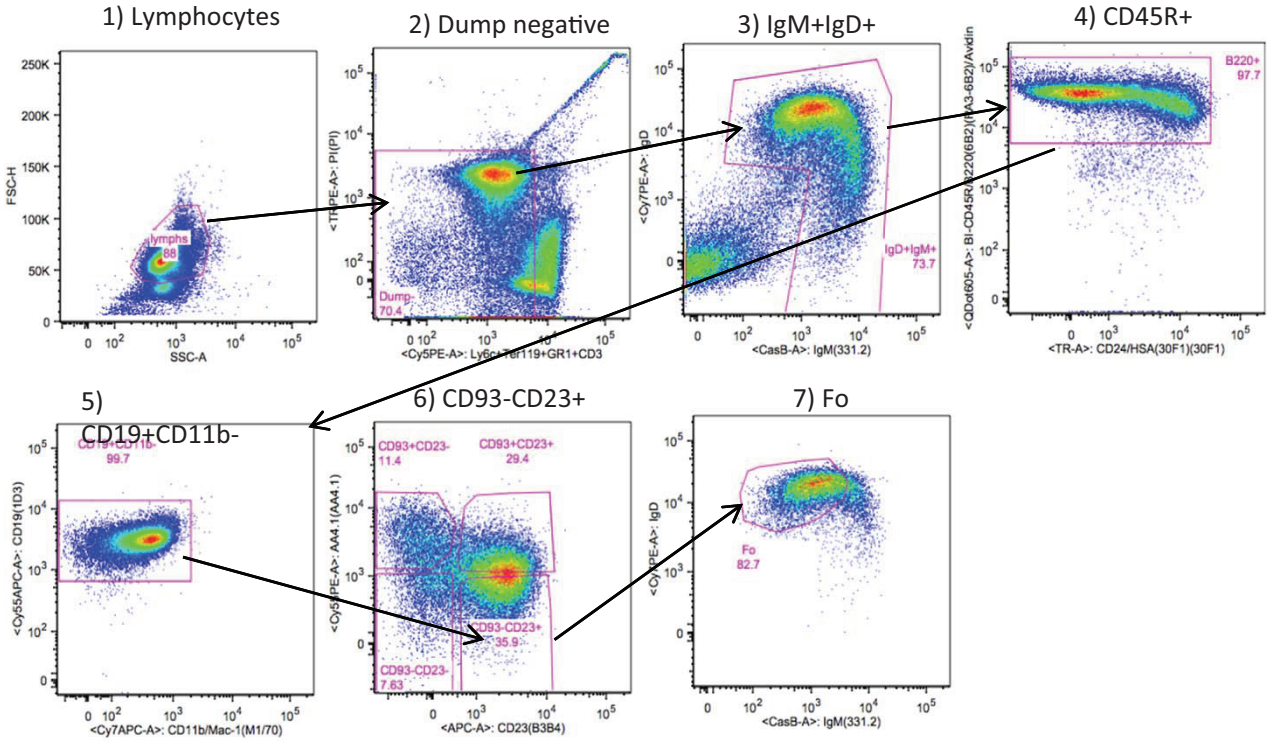
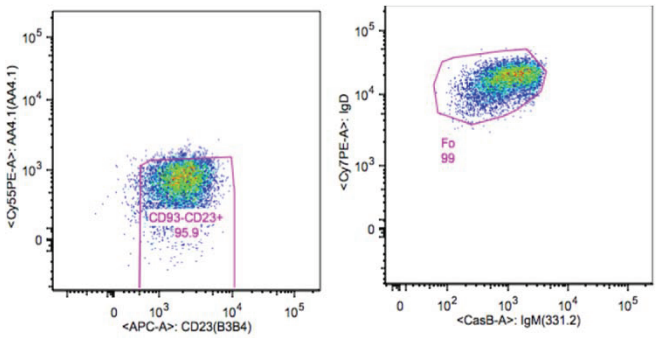
FOX CHASE CANCER CENTER

B.Fo.Sp: Splenic Follicular B Cells

Pre-sort



Post-sort



Tissue Preparation: Grind between slides, ACK lysis of RBCs
Time from Mouse to Trizol: 8:30AM-11:30AM

Antibody (Clone) - Fluorochrome:
CD43(S7)-FITC; CD21/35-PE; CD3(500A-A2),Ter119, Ly6c(AL21), GR1(8C5)-Cy5PE; CD93(AA4.1)-Cy55PE; IgD(JA12.5)-Cy7PE; CD23(B3B4)-APC; CD19(1D3)-Cy55APC; CD11b(Mac1)-Cy7APC; IgM(331.12)-Cascade Blue; CD45R(6B2)-biotin/Q Dot 605 Avidin

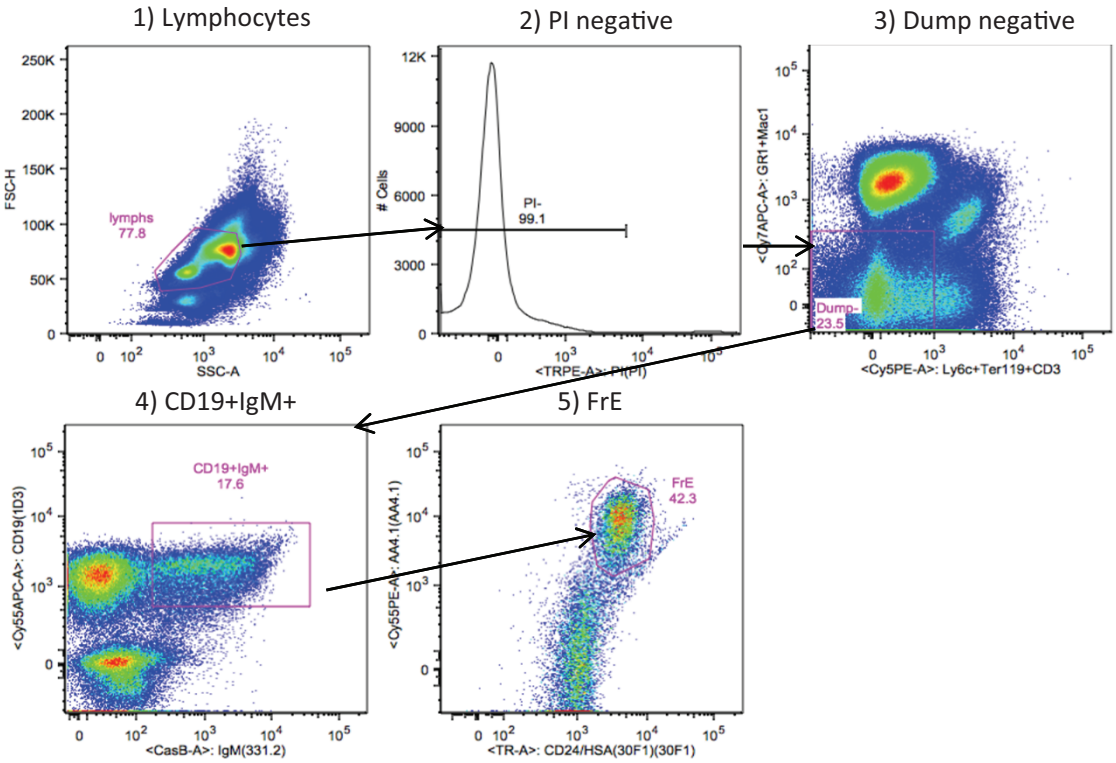
Follicular cells 99% purity

Immunological Genome Project

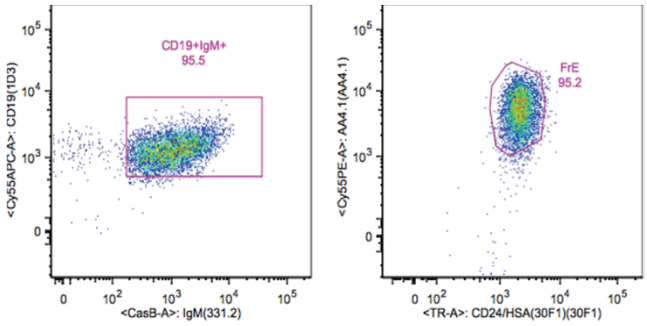
HARDY/HAYAKAWA LAB
FOX CHASE CANCER CENTER

B.FrE.BM: Bone marrow immature B cells (Fraction E)

Pre-sort



Post-sort



Tissue Preparation: Harvest cells from BM using 1ml syringe, ACK lysis of RBCs

Time from Mouse to Trizol: 8:30AM-1:30PM

Antibody (Clone) - Fluorochrome:

CD43(S7)-FITC; CD127(SB/199)-PE; CD3(500A-A2),Ter119, Ly6c(AL21); CD93(AA4.1)-Cy55PE; CD24(30F1)-Alexa594; CD117(2B8)-APC; CD19(1D3)-Cy55APC; GR1(8c5), CD11b(Mac1)-Cy7APC; IgM(331.12)-Cascade Blue; CD45R(6B2)-biotin/Q Dot 605 Avidin

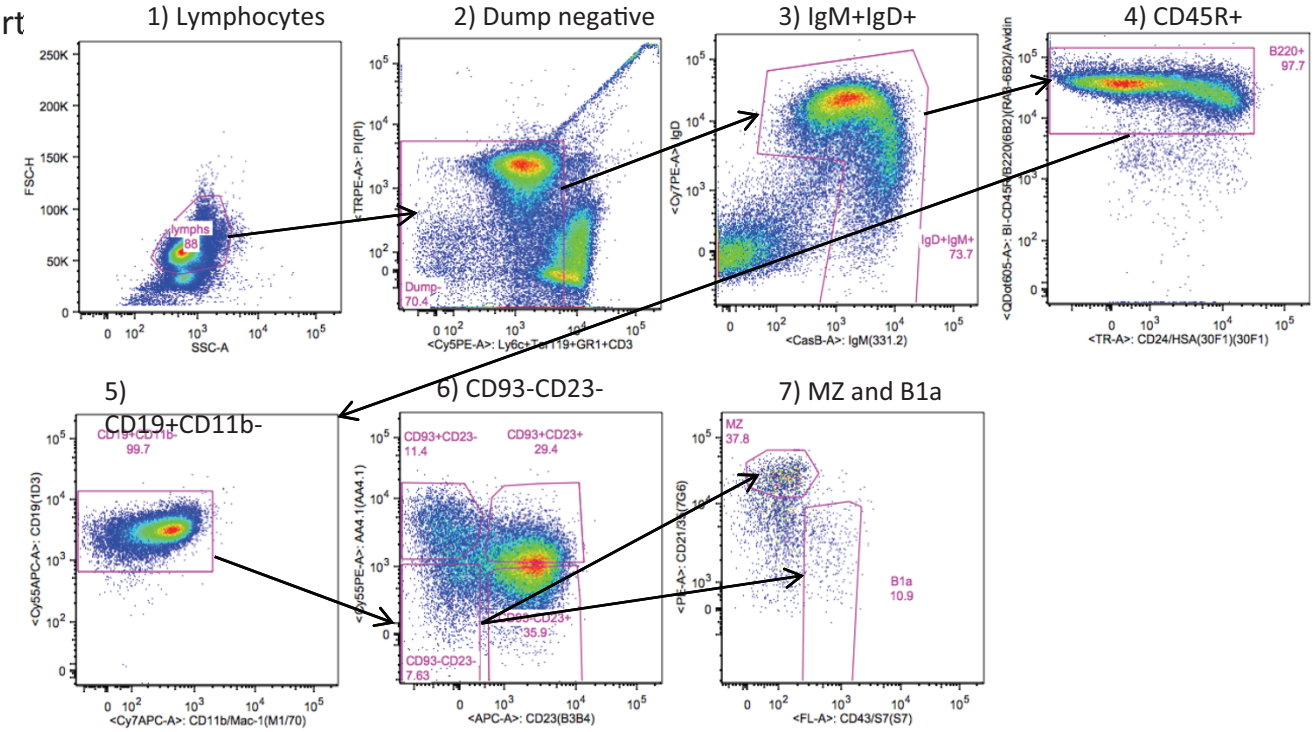
FrE cells 95.2% purity

Immunological Genome Project

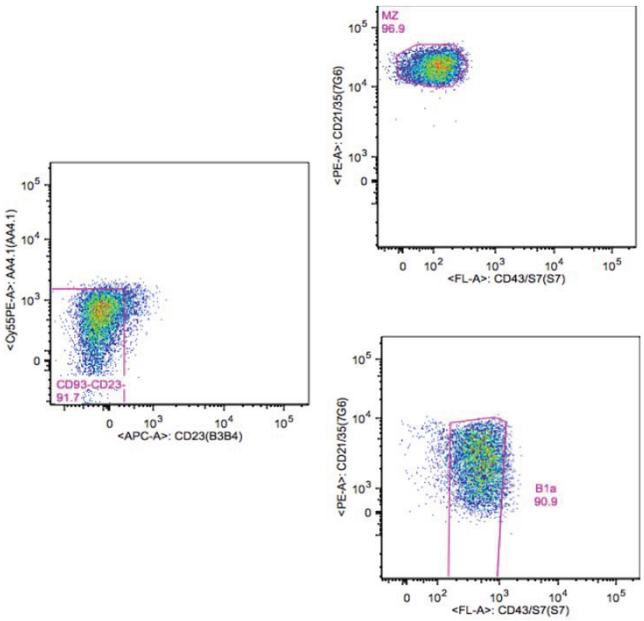
HARDY/HAYAKAWA LAB
FOX CHASE CANCER CENTER

B.MZ.Sp: Splenic Marginal Zone B Cells

Pre-sort



Post-sort



Tissue Preparation: Grind between slides, ACK lysis of RBCs
Time from Mouse to Trizol: 8:30AM-11:30AM

Antibody (Clone) - Fluorochrome:
CD43(S7)-FITC; CD21/35-PE; CD3(500A-A2),Ter119, Ly6c(AL21), GR1(8C5)-Cy5PE; CD93(AA4.1)-Cy55PE; IgD(JA12.5)-Cy7PE; CD23(B3B4)-APC; CD19(1D3)-Cy55APC; CD11b(Mac1)-Cy7APC; IgM(331.12)-Cascade Blue; CD45R(6B2)-biotin/Q Dot 605 Avidin

Marginal Zone cells 96.9% purity, B1a cells 90.9% purity

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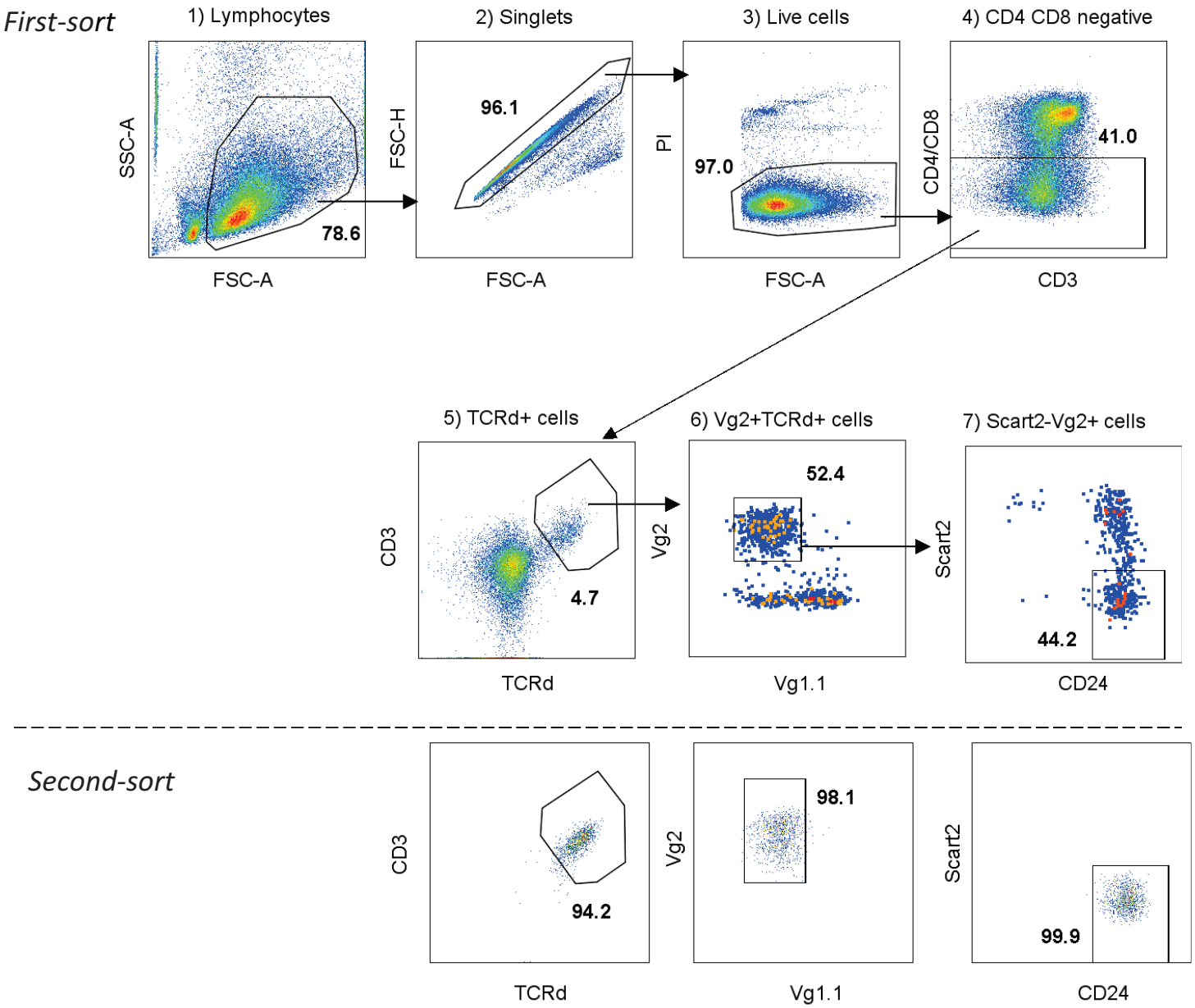
University of Massachusetts
Medical School

Tgd.g2+d1.24a+.Th: TCRvg2+ Scart2- immature thymocytes



Tissue Preparation: Glass Tissue Grinder

Time from Mouse to Trizol: 6:00AM-4:00PM

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) PE-Cy7
CD8(53-6.7) PE-Cy7
CD3e (145-2C11) PerCP-Cy5.5
TCRd (GL3) Brilliant Violet 421
Vg2 (UC3-10A6) FITC
Vg1.1 (2.11) APC
CD24 (M1/69) APC-eFluor780
Scart2 (25A2) Purified

gd.g2+d1.24a+.Th
99% purity

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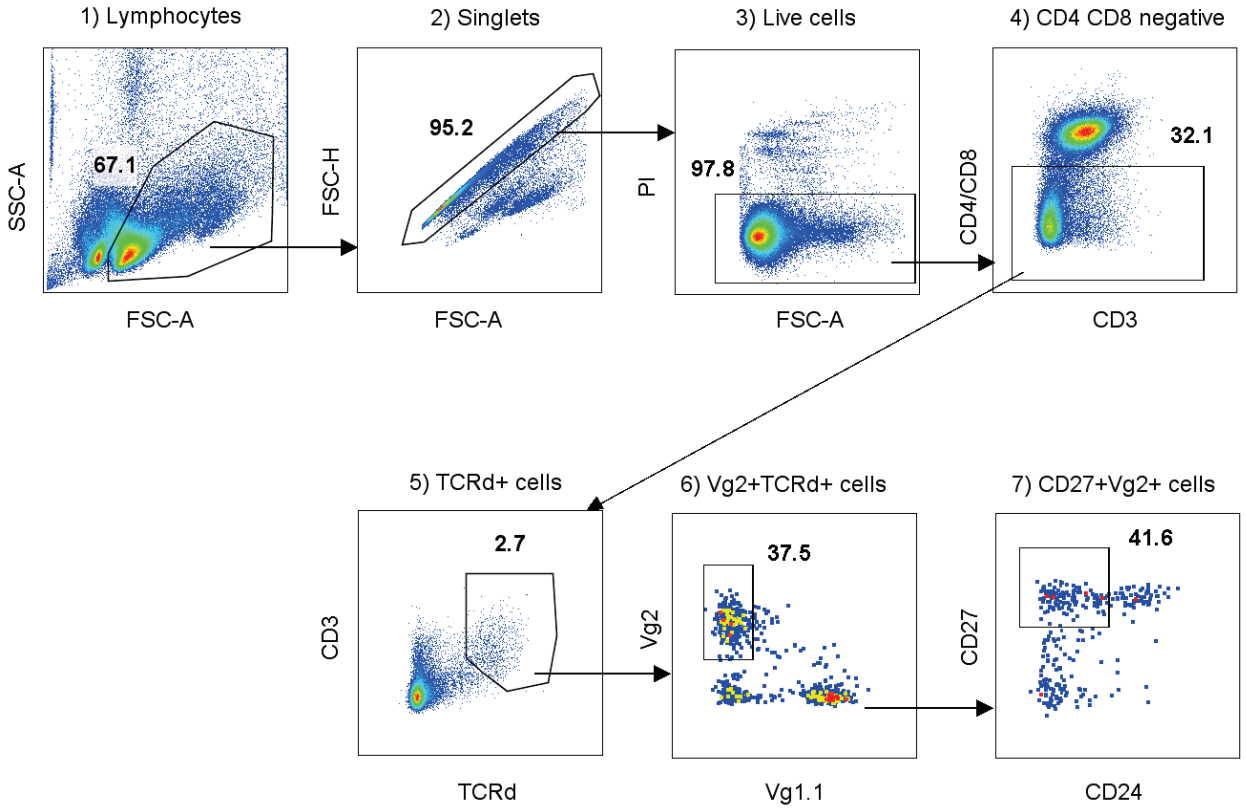
ImmGen.org

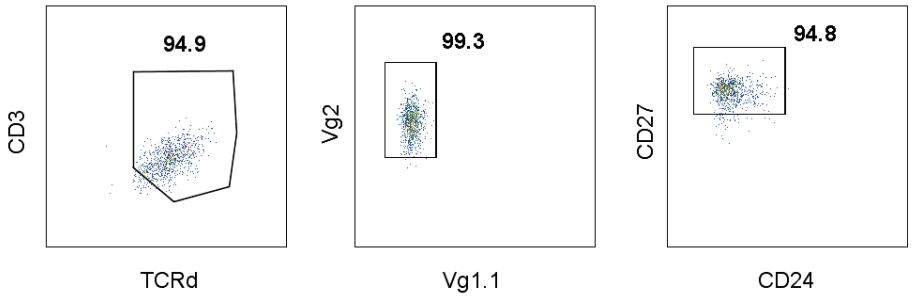
Kang Lab
University of Massachusetts Medical School

Tgd.g2+d1.LN: TCRvg2+ CD27+ lymphocytes

First-sort



Second-sort



Tissue Preparation: Glass Tissue Grinder

Time from Mouse to Trizol: 6:00AM-4:00PM

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) PE-Cy7
CD8(53-6.7) PE-Cy7
CD3e (145-2C11) PerCP-Cy5.5
TCRd (GL3) Brilliant Violet 421
Vg2 (UC3-10A6) FITC
Vg1.1 (2.11) APC
CD24 (M1/69) APC-eFluor780
CD27 (LG.7F9) PE

Tgd.g2+d1.LN
99% purity

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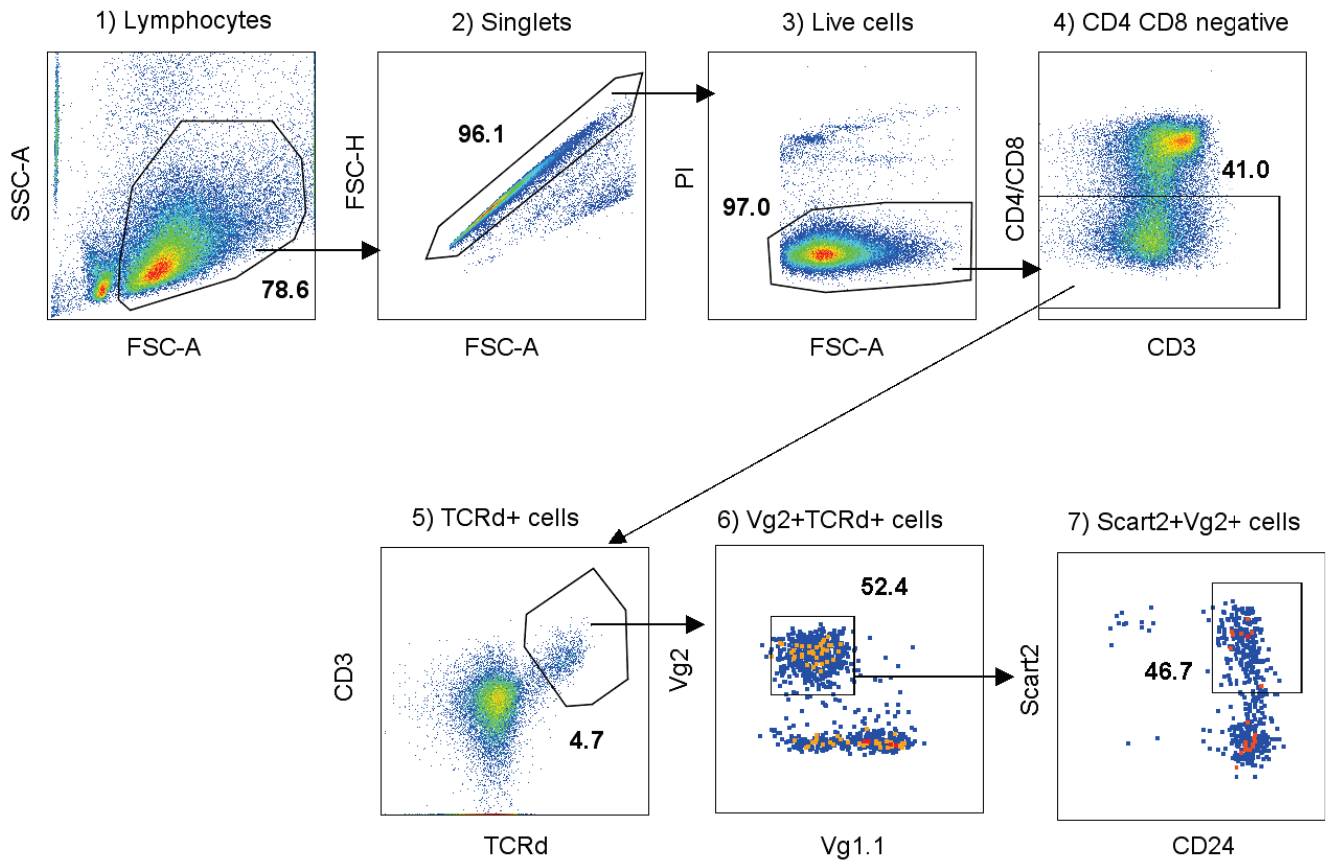
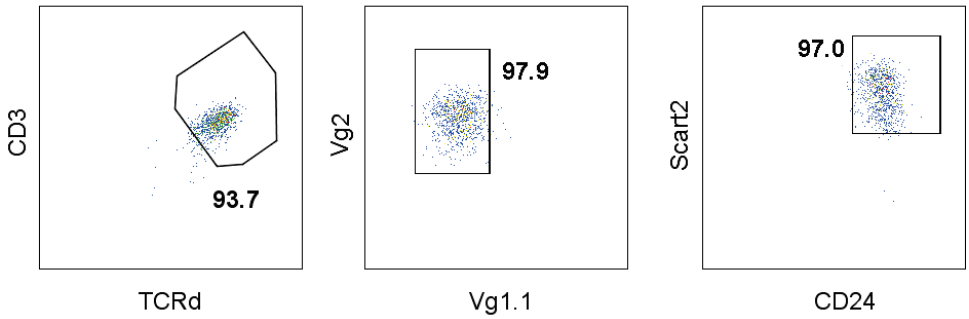
University of Massachusetts
Medical School

Tgd.g2+d17.24a+.Th: TCRvg2+ Scart2+ immature thymocytes

First-sort



Second-sort



Tissue Preparation: Glass Tissue Grinder

Time from Mouse to Trizol: 6:00AM-4:00PM

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) PE-Cy7
CD8(53-6.7) PE-Cy7
CD3e (145-2C11) PerCP-Cy5.5
TCRd (GL3) Brilliant Violet 421
Vg2 (UC3-10A6) FITC
Vg1.1 (2.11) APC
CD24 (M1/69) APC-eFluor780
Scart2 (25A2) Purified

Tgd.g2+d17.24a+.Th
99% purity

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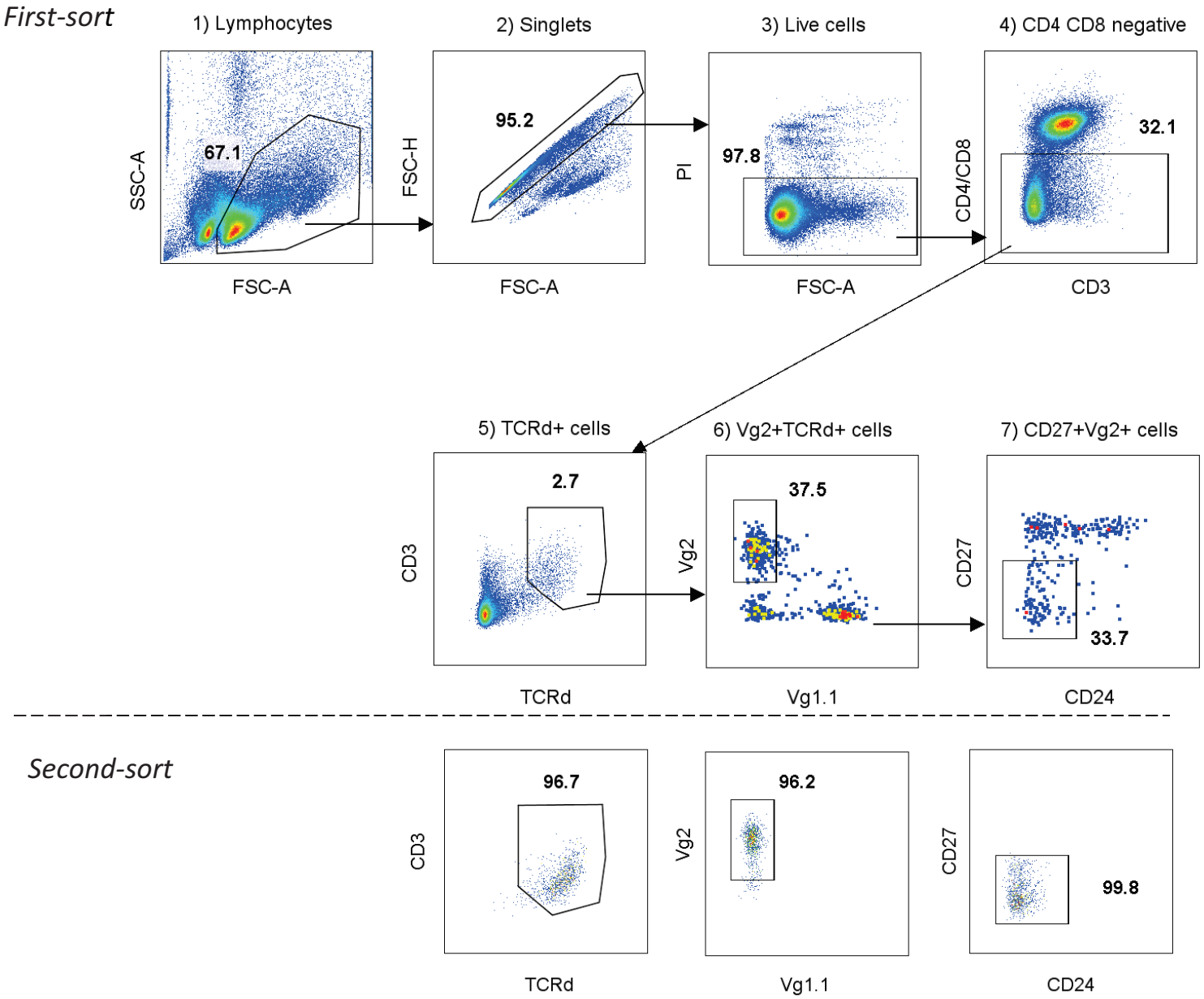
University of Massachusetts Medical School

Tgd.g2+d17.LN: TCRvg2+ CD27- lymphocytes



Tissue Preparation: Glass Tissue Grinder

Time from Mouse to Trizol: 6:00AM-4:00PM

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) PE-Cy7
CD8(53-6.7) PE-Cy7
CD3e (145-2C11) PerCP-Cy5.5
TCRd (GL3) Brilliant Violet 421
Vg2 (UC3-10A6) FITC
Vg1.1 (2.11) APC
CD24 (M1/69) APC-eFluor780
CD27 (LG.7F9) PE

Tgd.g2+d17.LN
99% purity

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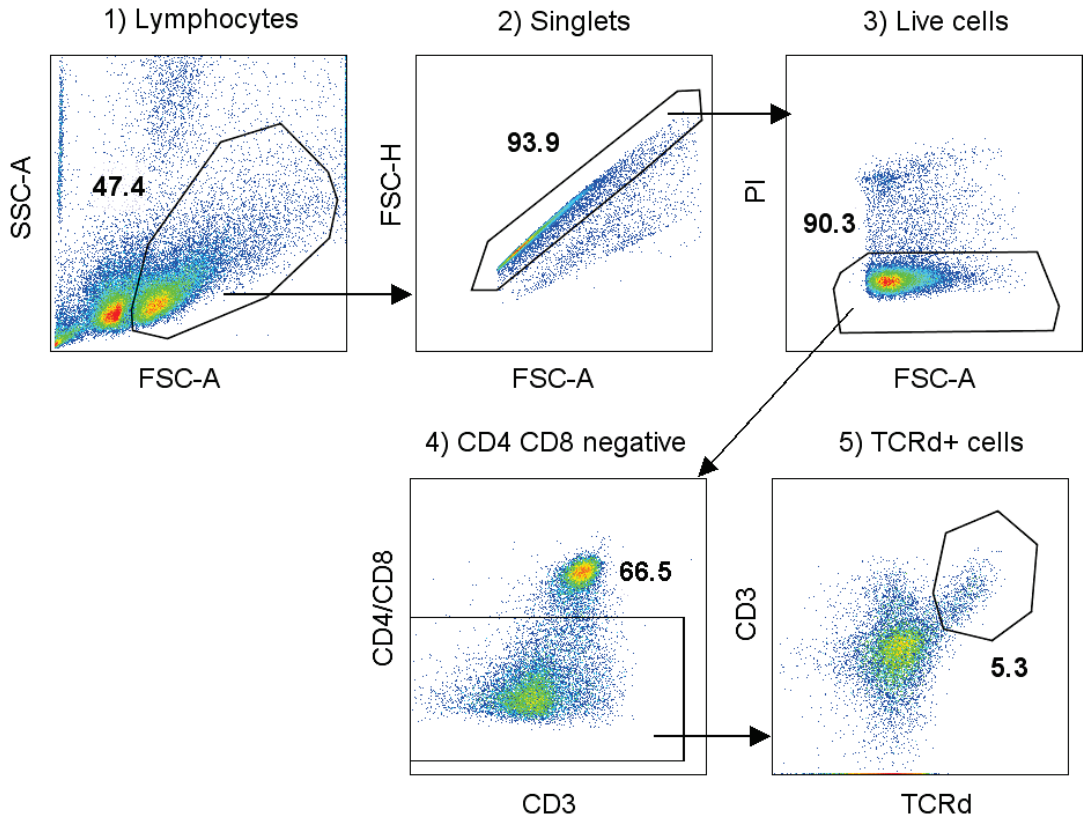
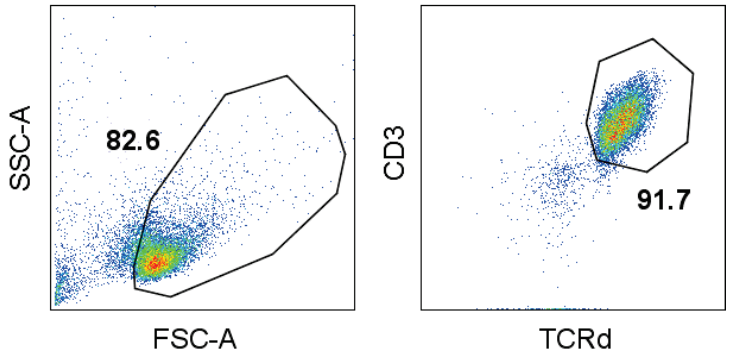
University of Massachusetts
Medical School

Tgd.Sp: Splenic gdT cells

First-sort



Second-sort



Tissue Preparation: Glass Tissue Grinder

Time from Mouse to Trizol: 6:00AM-4:00PM

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) PE-Cy7
CD8(53-6.7) PE-Cy7
CD3e (145-2C11) PerCP-Cy5.5
TCRd (GL3) Brilliant Violet 421

B6.Tgd.Sp
99% purity

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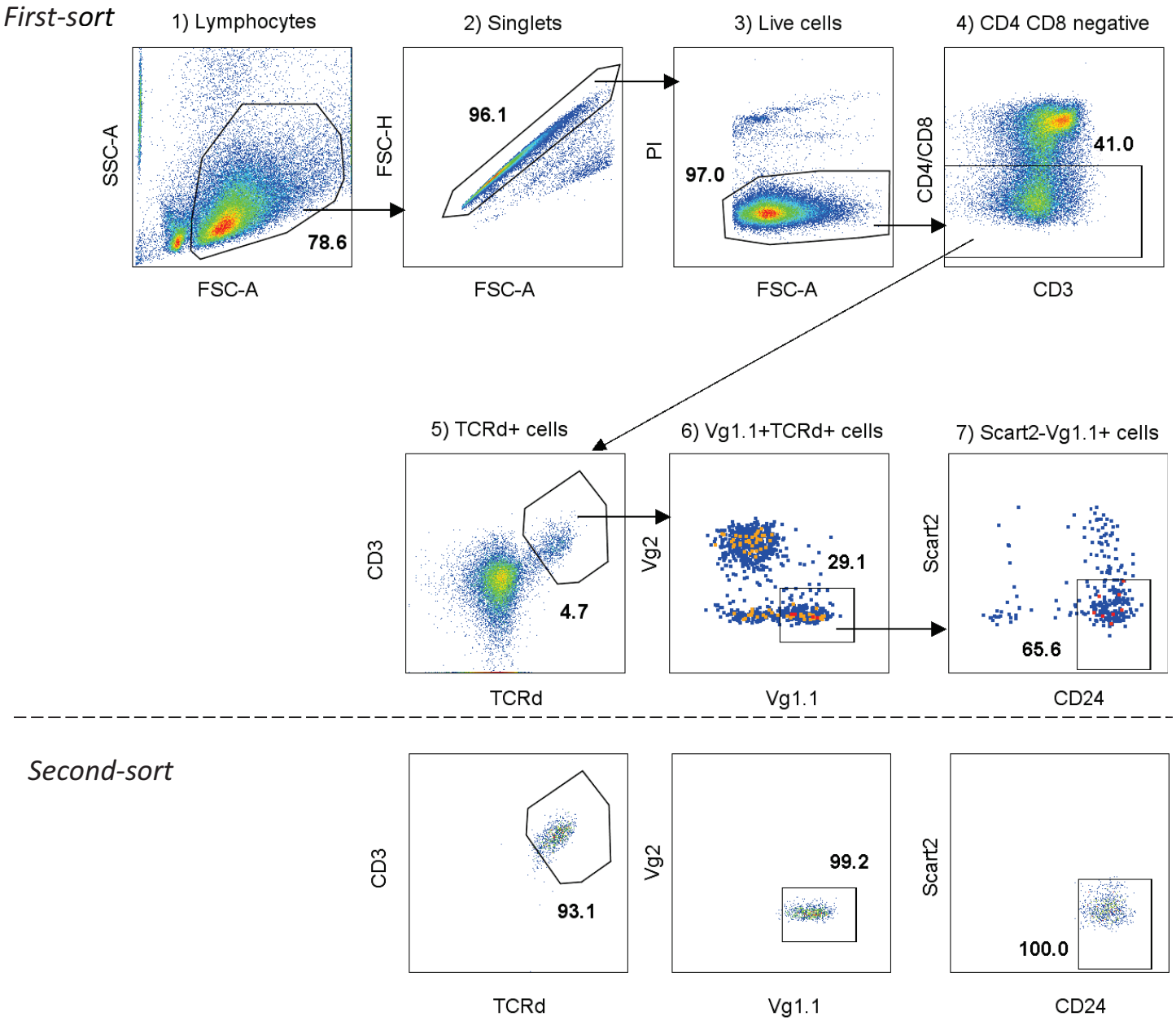
University of Massachusetts
Medical School

Tgd.g1.1+d1.24a+.Th: TCRvg1.1+ Scart2- immature thymocytes



Tissue Preparation: Glass Tissue Grinder

Time from Mouse to Trizol: 6:00AM-4:00PM

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) PE-Cy7
CD8(53-6.7) PE-Cy7
CD3e (145-2C11) PerCP-Cy5.5
TCRd (GL3) Brilliant Violet 421
Vg2 (UC3-10A6) FITC
Vg1.1 (2.11) APC
CD24 (M1/69) APC-eFluor780
Scart2 (25A2) Purified

gd.g1.1+d1.24a+.Th
99% purity

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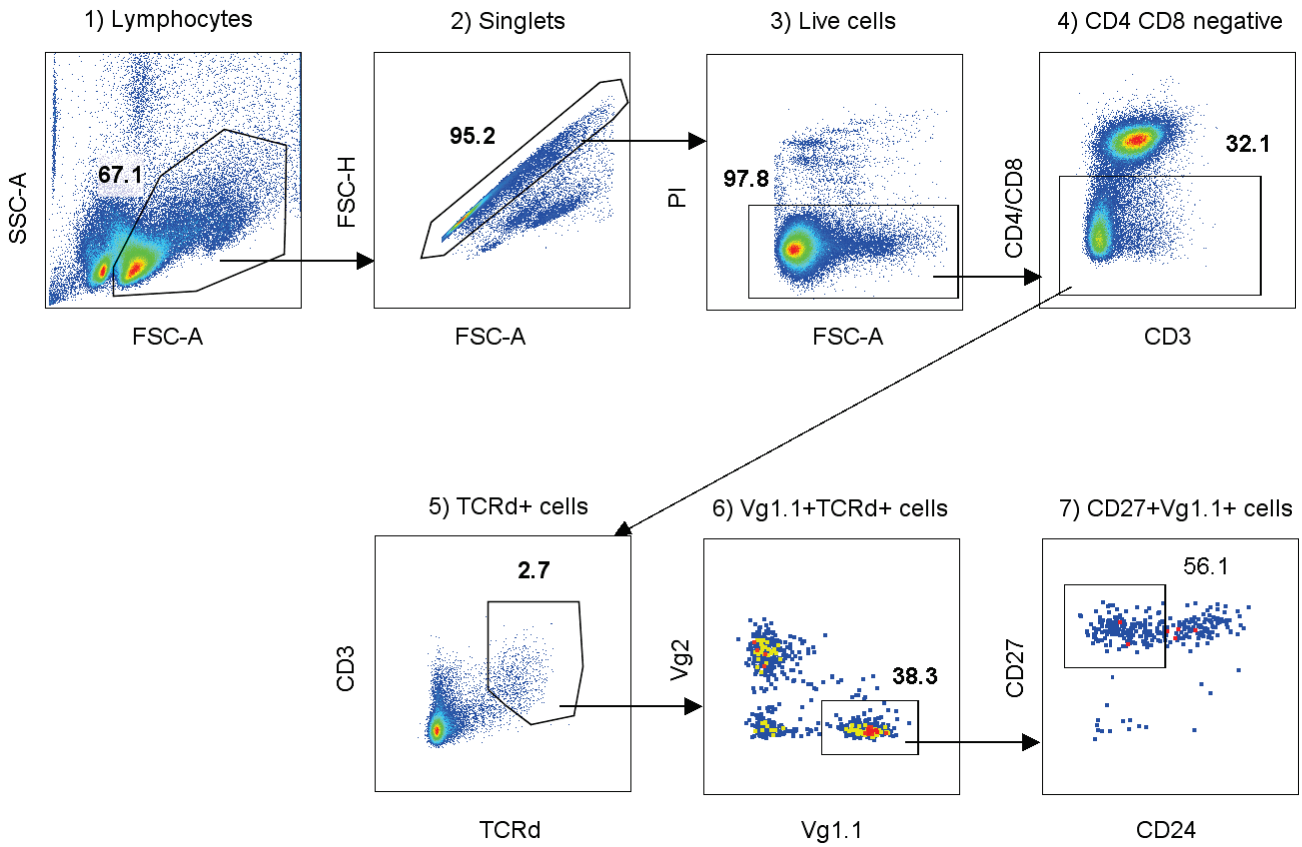
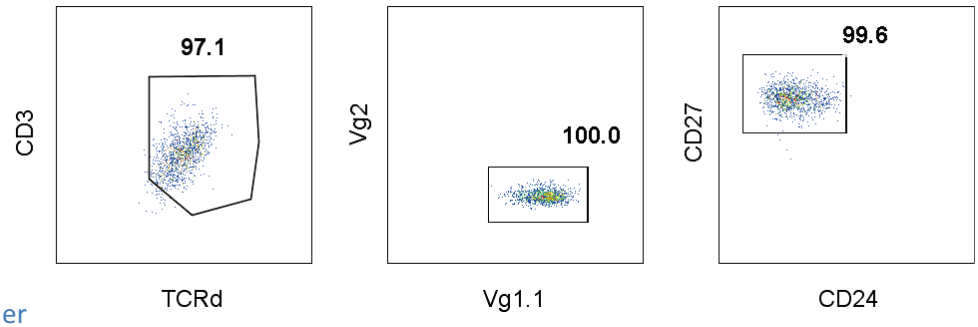
University of Massachusetts
Medical School

Tgd.g1.1+d1.LN: TCRvg1.1+ CD27+ lymphocytes

First-sort



Second-sort



Tissue Preparation: Glass Tissue Grinder

Time from Mouse to Trizol: 6:00AM-4:00PM

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) PE-Cy7
CD8(53-6.7) PE-Cy7
CD3e (145-2C11) PerCP-Cy5.5
TCRd (GL3) Brilliant Violet 421
Vg2 (UC3-10A6) FITC
Vg1.1 (2.11) APC
CD24 (M1/69) APC-eFluor780
CD27 (LG.7F9) PE

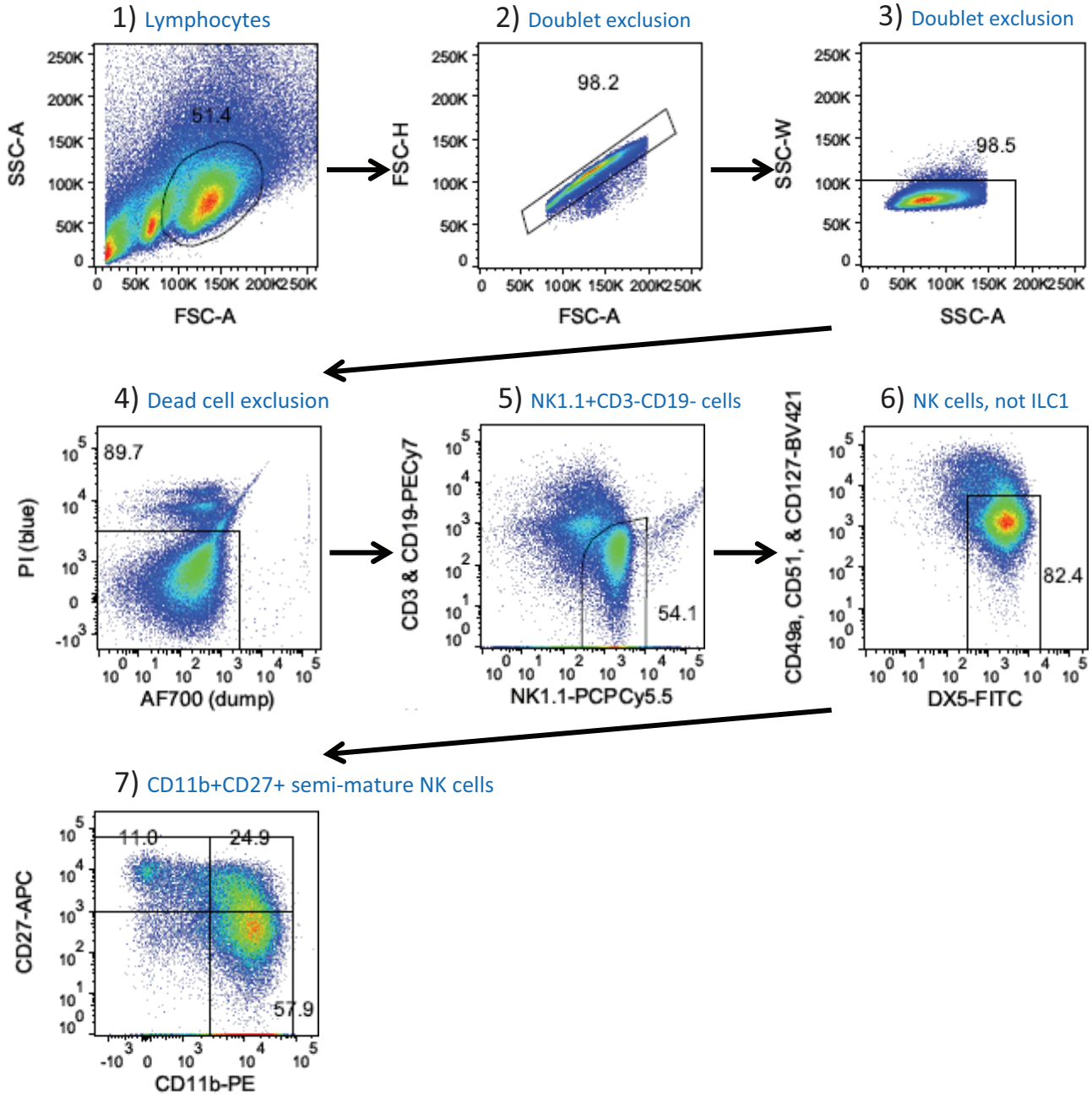
Tgd.g1.1+d1.LN
99% purity

Immunological Genome Project
ImmGen.org

[Tsukasa Nabekura]
Lewis Lanier lab
UCSF

NK.27+11b+.Sp: Splenic semi-mature NK cells

Pre-sort

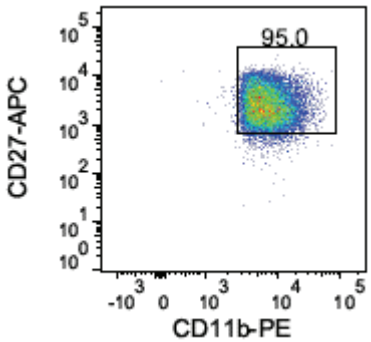


Tissue Preparation: Splenocytes
Time from Mouse to TCL buffer (1% 2-ME) or BAMBANKER:
7:00 am – 2-3:00 pm

Antibody (Clone) - Fluorochrome:

- CD49b (DX5) – FITC
- NK1.1 (PK136) – PerCP-Cy5.5
- CD3e (145-2C11) – PE-Cy7
- CD19 (1D3) – PE-Cy7
- CD11b (M1/70) – PE
- CD27 (LG.3A10) – APC
- CD49a (REA493) – Biotin
- CD127 (A7R34) – Biotin
- CD51 (RMV-7) – Biotin
- Streptavidin-BV421

Post-sort



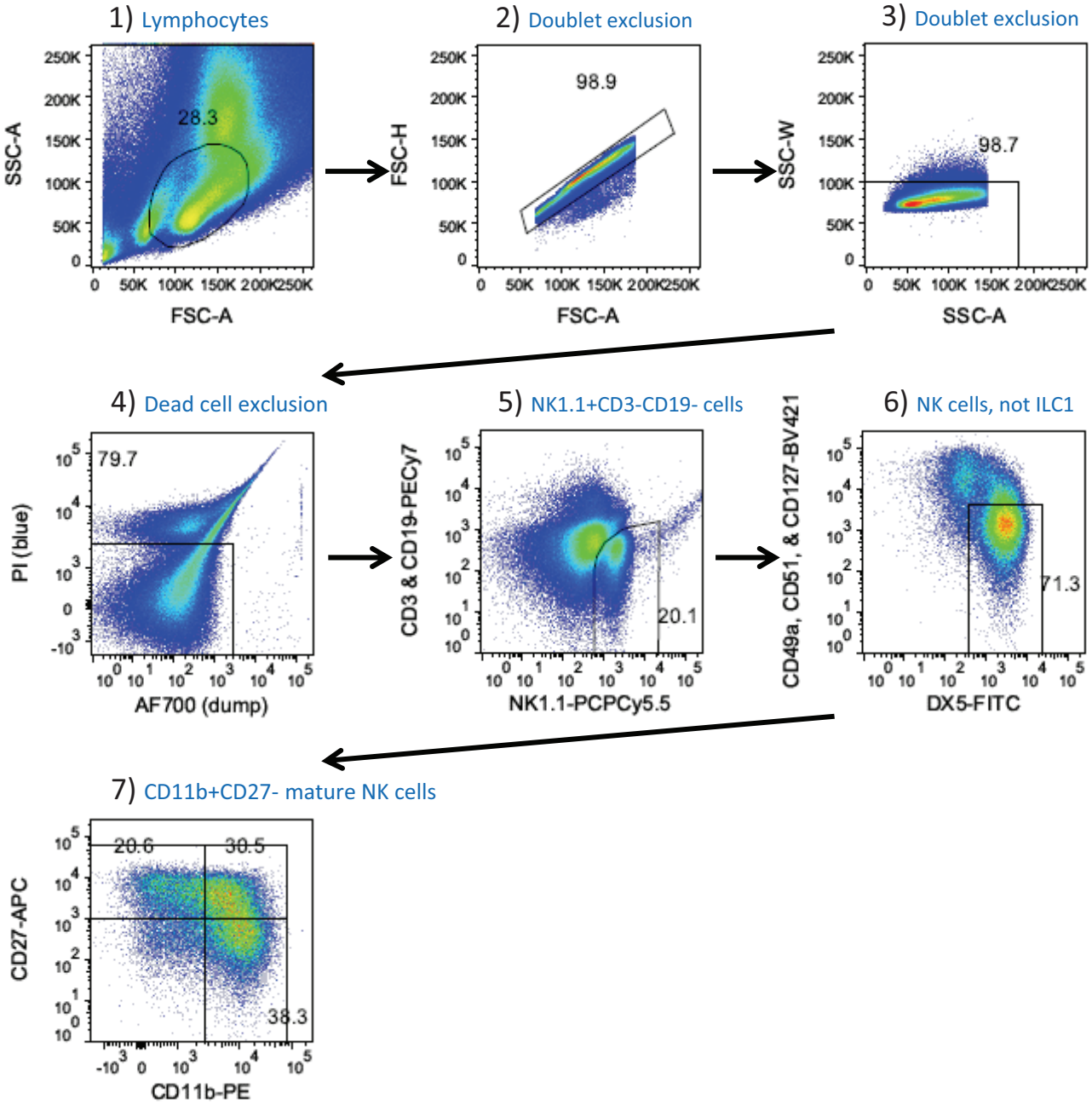
FILL-IN cells
95.0 % purity

Immunological Genome Project
ImmGen.org

[Tsukasa Nabekura]
Lewis Lanier lab
UCSF

NK.27-11b+.BM: Bone marrow mature NK cells

Pre-sort

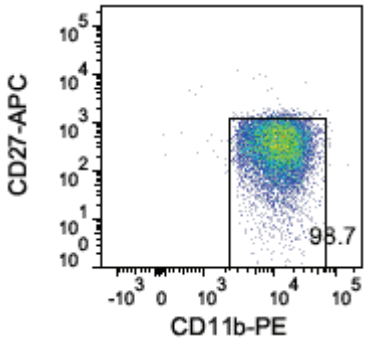


Tissue Preparation: Splenocytes
Time from Mouse to TCL buffer (1% 2-ME) or BAMBANKER:
7:00 am – 2-3:00 pm

Antibody (Clone) - Fluorochrome:

- CD49b (DX5) – FITC
- NK1.1 (PK136) – PerCP-Cy5.5
- CD3e (145-2C11) – PE-Cy7
- CD19 (1D3) – PE-Cy7
- CD11b (M1/70) – PE
- CD27 (LG.3A10) – APC
- CD49a (REA493) – Biotin
- CD127 (A7R34) – Biotin
- CD51 (RMV-7) – Biotin
- Streptavidin-BV421

Post-sort



FILL-IN cells
98.7% purity

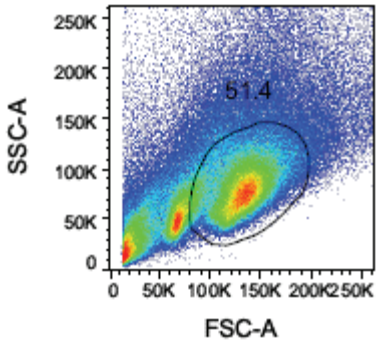
Immunological Genome Project
ImmGen.org

[Tsukasa Nabekura]
Lewis Lanier lab
UCSF

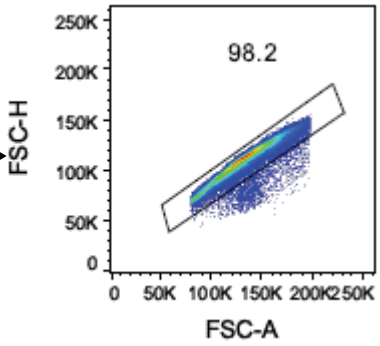
NK.27-11b+.Sp: Splenic mature NK cells

Pre-sort

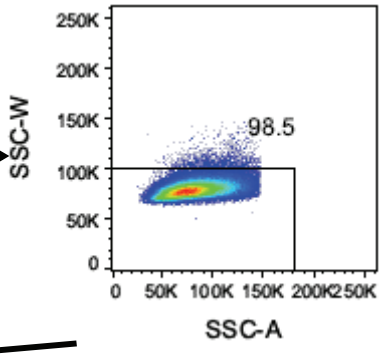
1) Lymphocytes



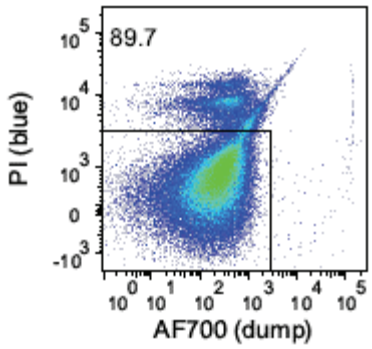
2) Doublet exclusion



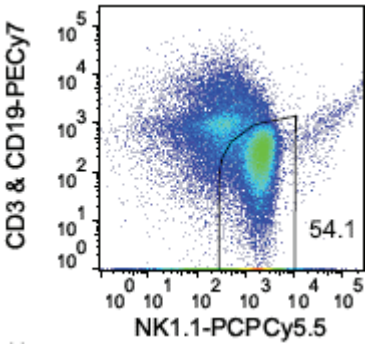
3) Doublet exclusion



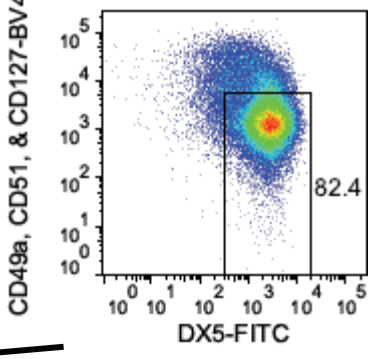
4) Dead cell exclusion



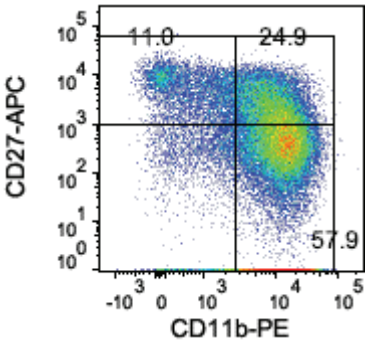
5) NK1.1+CD3-CD19- cells



6) NK cells, not ILC1



7) CD11b+CD27- mature NK cells

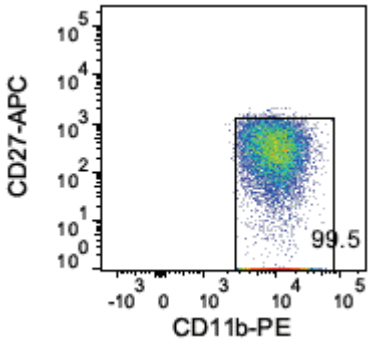


Tissue Preparation: Splenocytes
Time from Mouse to TCL buffer (1% 2-ME) or BAMBANKER:
7:00 am – 2-3:00 pm

Antibody (Clone) - Fluorochrome:

- CD49b (DX5) – FITC
- NK1.1 (PK136) – PerCP-Cy5.5
- CD3e (145-2C11) – PE-Cy7
- CD19 (1D3) – PE-Cy7
- CD11b (M1/70) – PE
- CD27 (LG.3A10) – APC
- CD49a (REA493) – Biotin
- CD127 (A7R34) – Biotin
- CD51 (RMV-7) – Biotin
- Streptavidin-BV421

Post-sort



FILL-IN cells
99.5 % purity

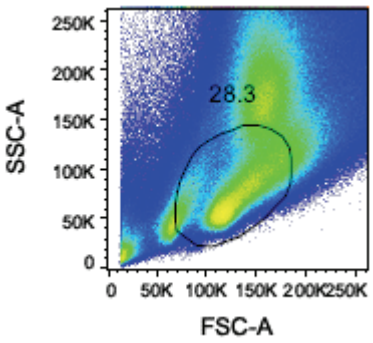
Immunological Genome Project
ImmGen.org

[Tsukasa Nabekura]
Lewis Lanier lab
UCSF

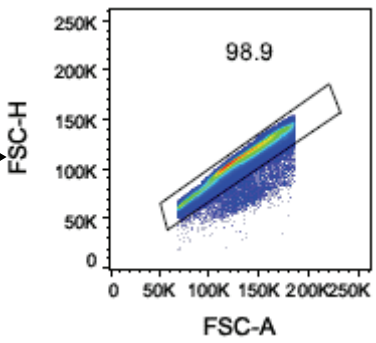
NK.27+11b-.BM: Bone marrow immature NK cells

Pre-sort

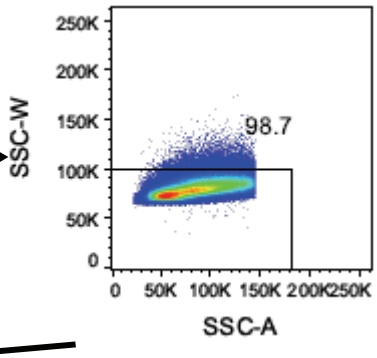
1) Lymphocytes



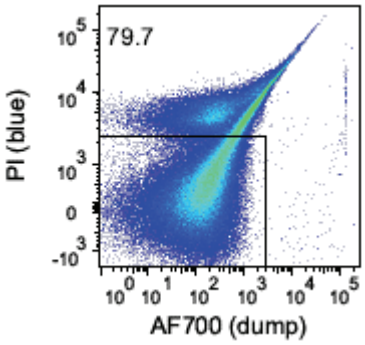
2) Doublet exclusion



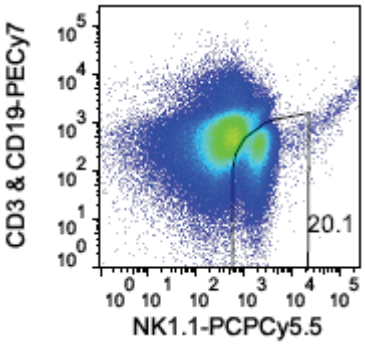
3) Doublet exclusion



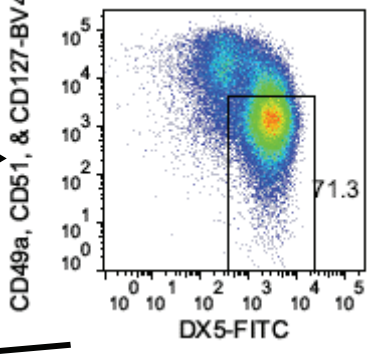
4) Dead cell exclusion



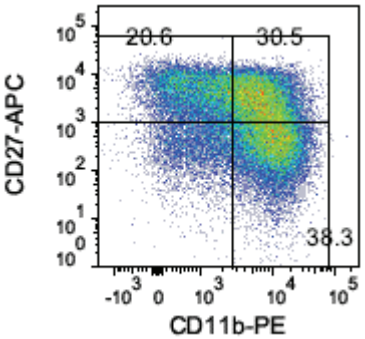
5) NK1.1+CD3-CD19- cells



6) NK cells, not ILC1



7) CD11b-CD27+ immature NK cells

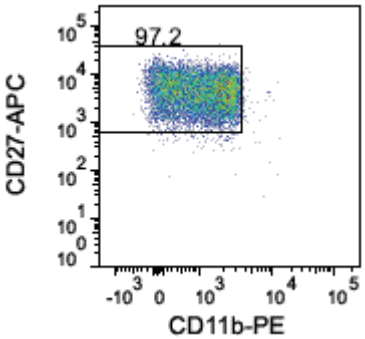


Tissue Preparation: Splenocytes
Time from Mouse to TCL buffer (1% 2-ME) or BAMBANKER:
7:00 am – 2-3:00 pm

Antibody (Clone) - Fluorochrome:

- CD49b (DX5) – FITC
- NK1.1 (PK136) – PerCP-Cy5.5
- CD3e (145-2C11) – PE-Cy7
- CD19 (1D3) – PE-Cy7
- CD11b (M1/70) – PE
- CD27 (LG.3A10) – APC
- CD49a (REA493) – Biotin
- CD127 (A7R34) – Biotin
- CD51 (RMV-7) – Biotin
- Streptavidin-BV421

Post-sort



FILL-IN cells
97.2% purity

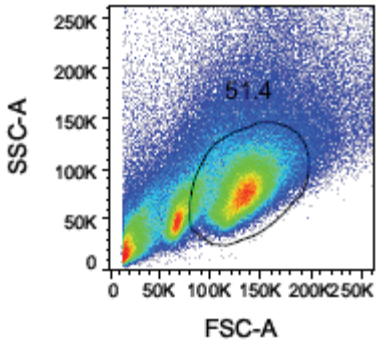
Immunological Genome Project
ImmGen.org

[Tsukasa Nabekura]
Lewis Lanier lab
UCSF

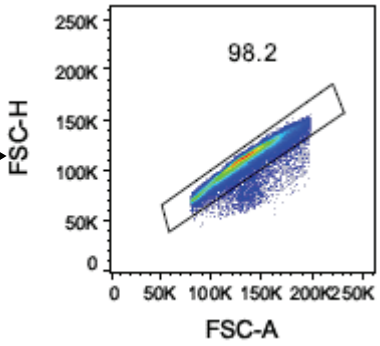
NK.27+11b-.Sp: Splenic immature NK cells

Pre-sort

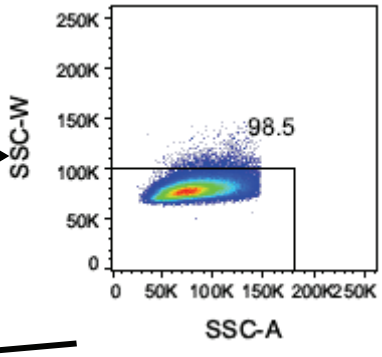
1) Lymphocytes



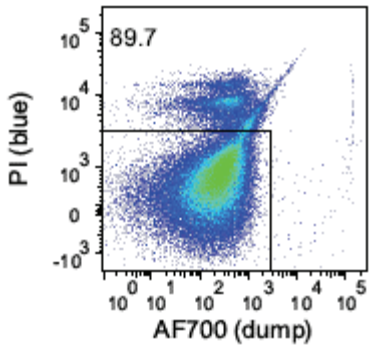
2) Doublet exclusion



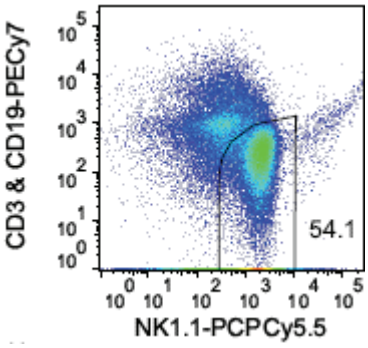
3) Doublet exclusion



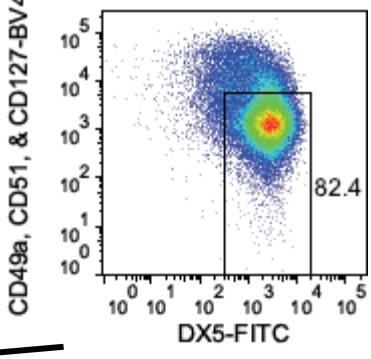
4) Dead cell exclusion



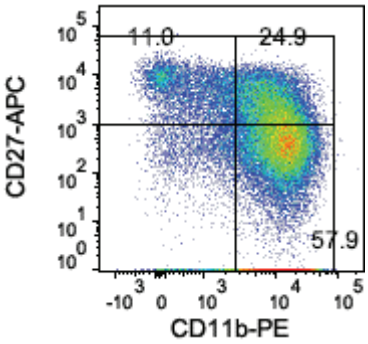
5) NK1.1+CD3-CD19- cells



6) NK cells, not ILC1



7) CD11b-CD27+ immature NK cells

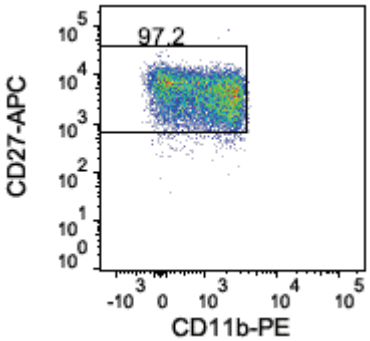


Tissue Preparation: Splenocytes
Time from Mouse to TCL buffer (1% 2-ME) or BAMBANKER:
7:00 am – 2-3:00 pm

Antibody (Clone) - Fluorochrome:

- CD49b (DX5) – FITC
- NK1.1 (PK136) – PerCP-Cy5.5
- CD3e (145-2C11) – PE-Cy7
- CD19 (1D3) – PE-Cy7
- CD11b (M1/70) – PE
- CD27 (LG.3A10) – APC
- CD49a (REA493) – Biotin
- CD127 (A7R34) – Biotin
- CD51 (RMV-7) – Biotin
- Streptavidin-BV421

Post-sort



FILL-IN cells
97.2% purity

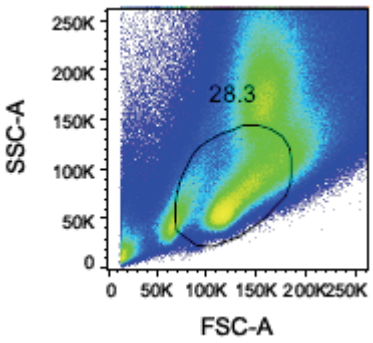
Immunological Genome Project
ImmGen.org

[Tsukasa Nabekura]
Lewis Lanier lab
UCSF

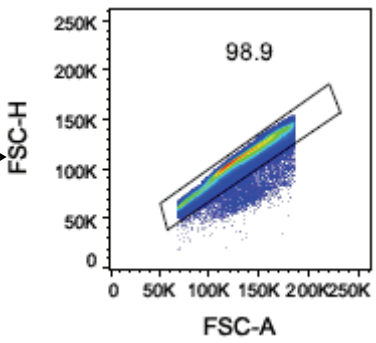
NK.27+11b+.BM: Bone marrow semi-mature NK cells

Pre-sort

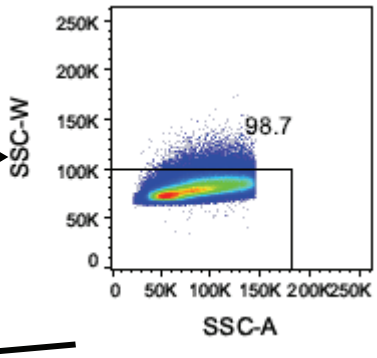
1) Lymphocytes



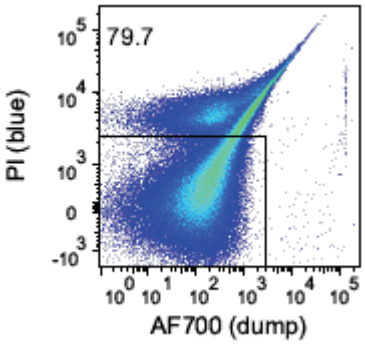
2) Doublet exclusion



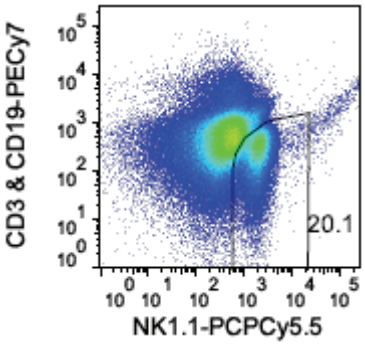
3) Doublet exclusion



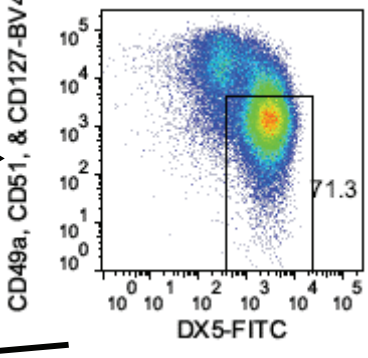
4) Dead cell exclusion



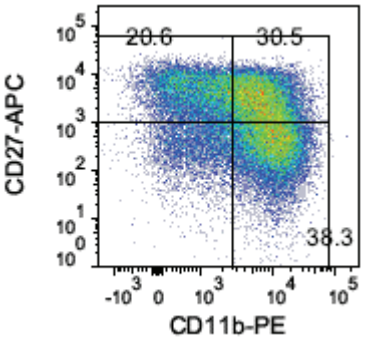
5) NK1.1+CD3-CD19- cells



6) NK cells, not ILC1



7) CD11b+CD27+ semi-mature NK cells

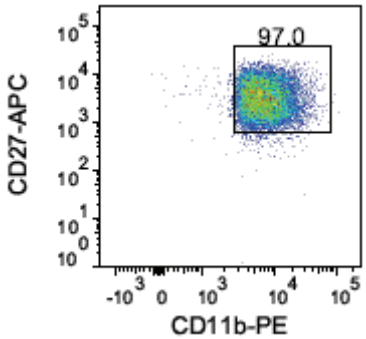


Tissue Preparation: Splenocytes
Time from Mouse to TCL buffer (1% 2-ME) or BAMBANKER:
7:00 am – 2-3:00 pm

Antibody (Clone) - Fluorochrome:

- CD49b (DX5) – FITC
- NK1.1 (PK136) – PerCP-Cy5.5
- CD3e (145-2C11) – PE-Cy7
- CD19 (1D3) – PE-Cy7
- CD11b (M1/70) – PE
- CD27 (LG.3A10) – APC
- CD49a (REA493) – Biotin
- CD127 (A7R34) – Biotin
- CD51 (RMV-7) – Biotin
- Streptavidin-BV421

Post-sort



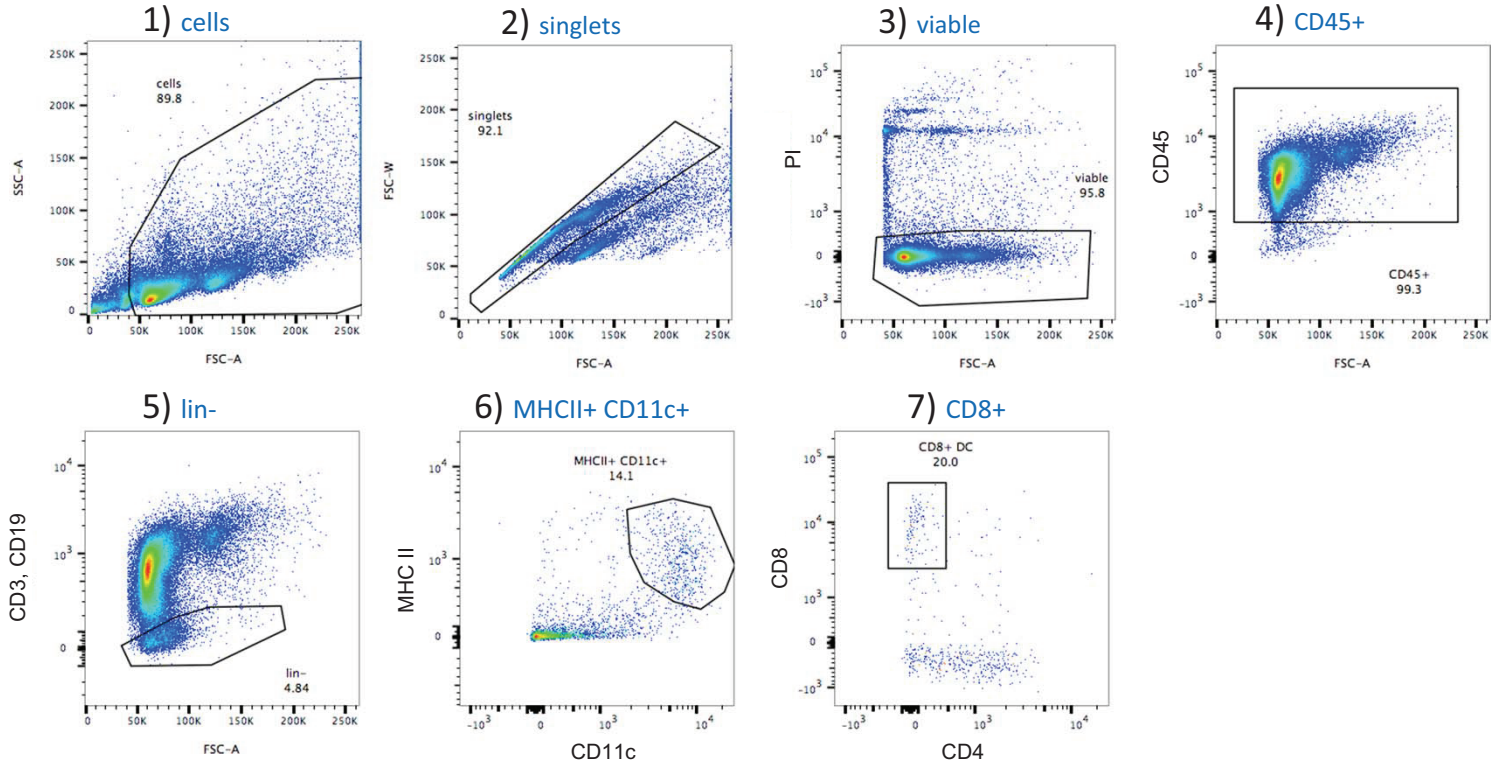
FILL-IN cells
97.0% purity

Immunological Genome Project
ImmGen.org

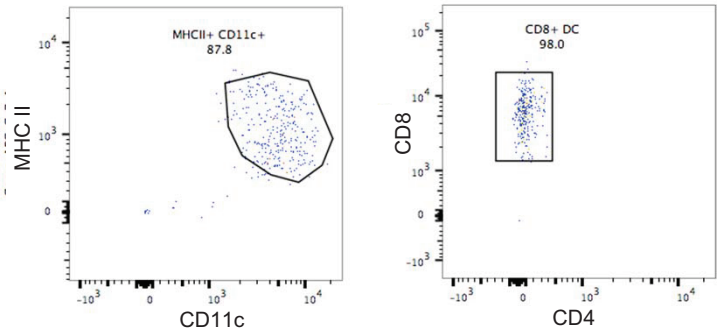
[Barbara Maier]
Miriam Merad Lab
Mount Sinai School of Medicine

DC.8+.Sp: Splenic CD8+ Dendritic cells

Pre-sort



Post-sort



Tissue Preparation: Collagenase V digestion, push through 70um cell strainer

Time from Mouse to Trizol: 09:00am-12:00pm

Antibody (Clone) - Fluorochrome:

- CD3, CD19 – FITC
- CD8 – PE
- CD4 – APC
- CD11c – PE/Cy7
- CD45 – BV510
- MHC II – APC/Cy7
- viability - PI

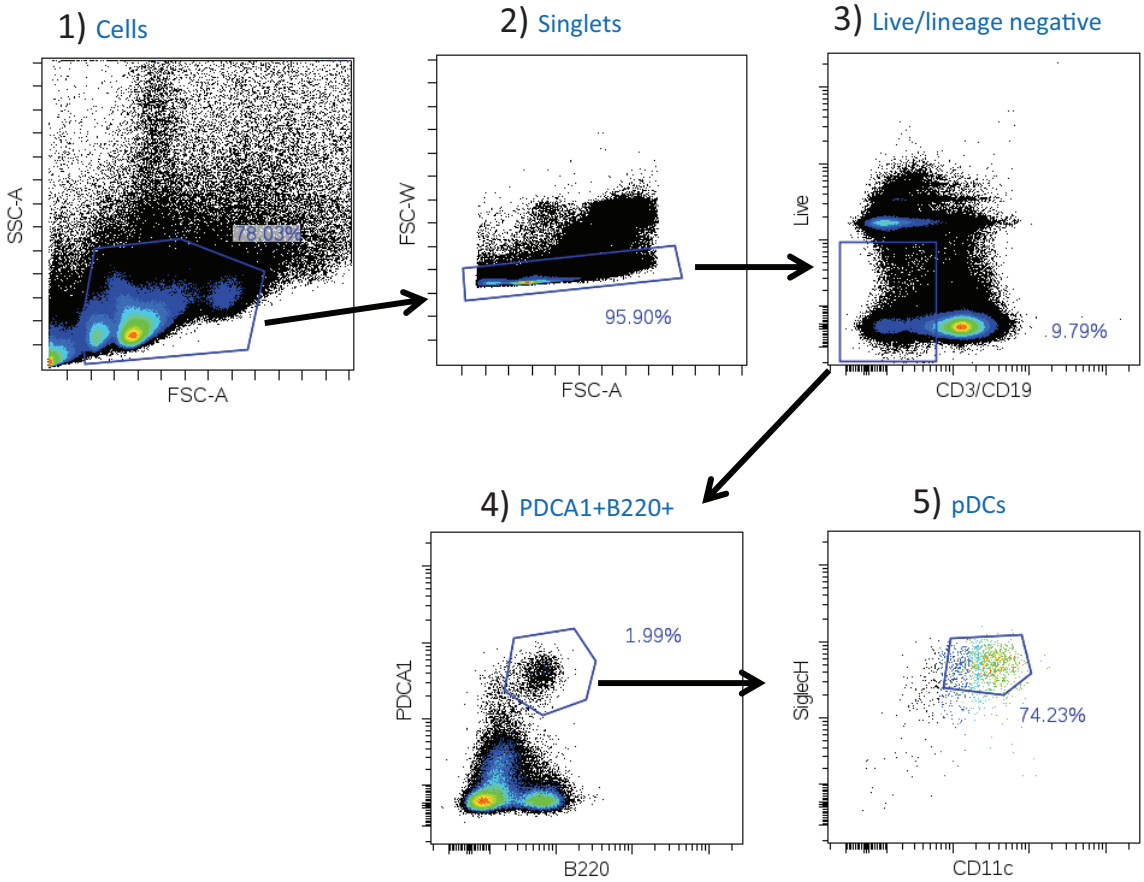
10000 cells
98.0% purity

Immunological Genome Project
ImmGen.org

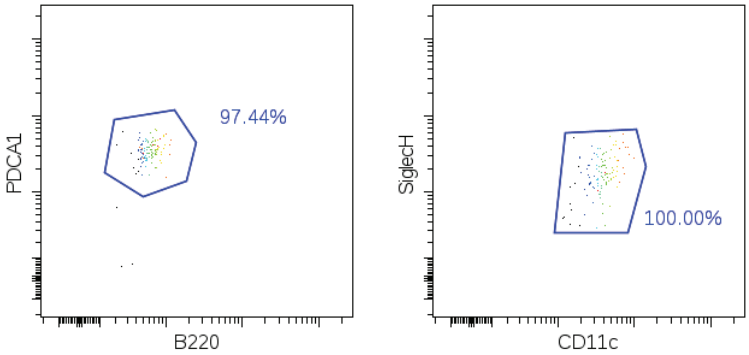
Brown/Merad Lab
Icahn School of Medicine at Mount Sinai

DC.pDC.Sp: Splenic plasmacytoid dendritic cells

Pre-sort



Post-sort



Tissue Preparation: Filter/syringe plunger teasing

Time from Mouse to Trizol: 09:30am-01:00pm

Antibody (Clone) - Fluorochrome:
CD3 (eBio500A2)
CD19 (eBio1D3) FITC
CD11c (N418) PE
B220 (RA3-6B2) eFluor450
PDCA1 (eBio927) APC
Siglec H (eBio440c) PE-Cy7

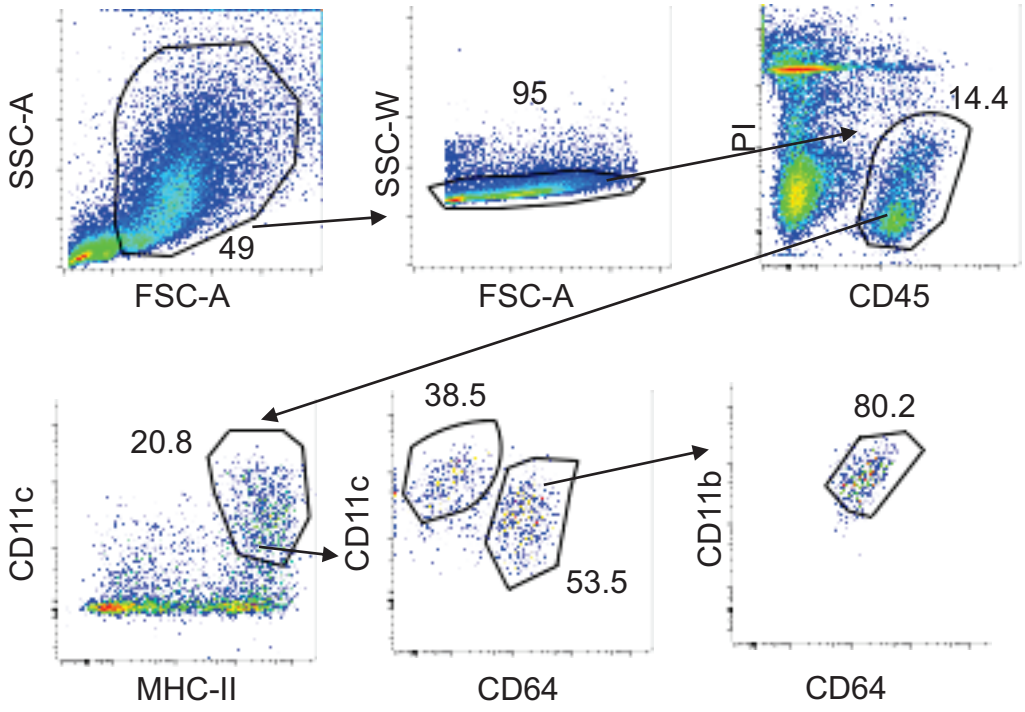
~9,000 cells
100% purity

Immunological Genome Project
ImmGen.org

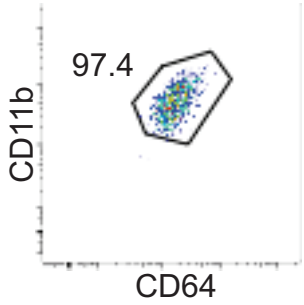
Merad Lab
Mount Sinai

MF.LP.SI: Small intestine lamina propria macrophages

Pre-sort



Second Sort



Tissue Preparation: Collagenase DNAse

Time from Mouse to Trizol: 9:00 am-2:00pm

Antibody (Clone) - Fluorochrome:

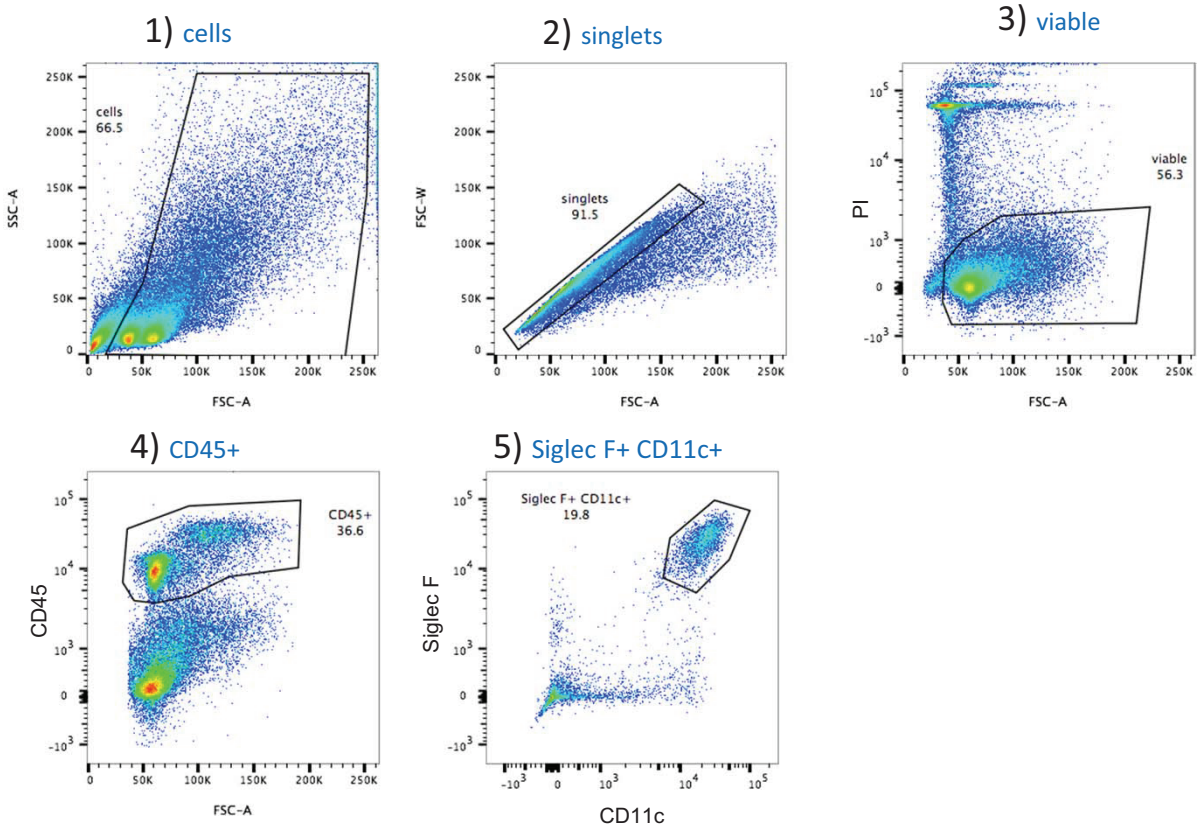
CD45 -- APC-Cy7
CD11c -- PE-Cy7
MHCII -- PacificBlue
CD64 -- APC
CD103 -- PE
CD11b -- FITC

Immunological Genome Project
ImmGen.org

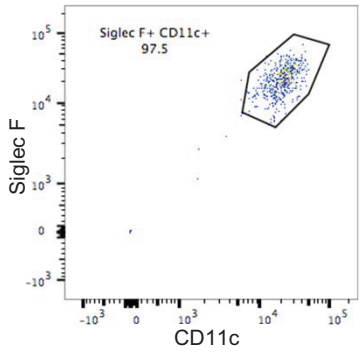
[Barbara Maier]
Miriam Merad Lab
Mount Sinai School of Medicine

MF.Alv.Lu: Lung alveolar macrophages

Pre-sort



Post-sort



Tissue Preparation: Collagenase V digestion, dounce glass homogenizer

Time from Mouse to Trizol: 09:00am-12:00am

Antibody (Clone) - Fluorochrome:

- Siglec F – PE
- CD11c (N418) – PE/Cy7
- CD45 (30-F11) – BV510
- viability - PI

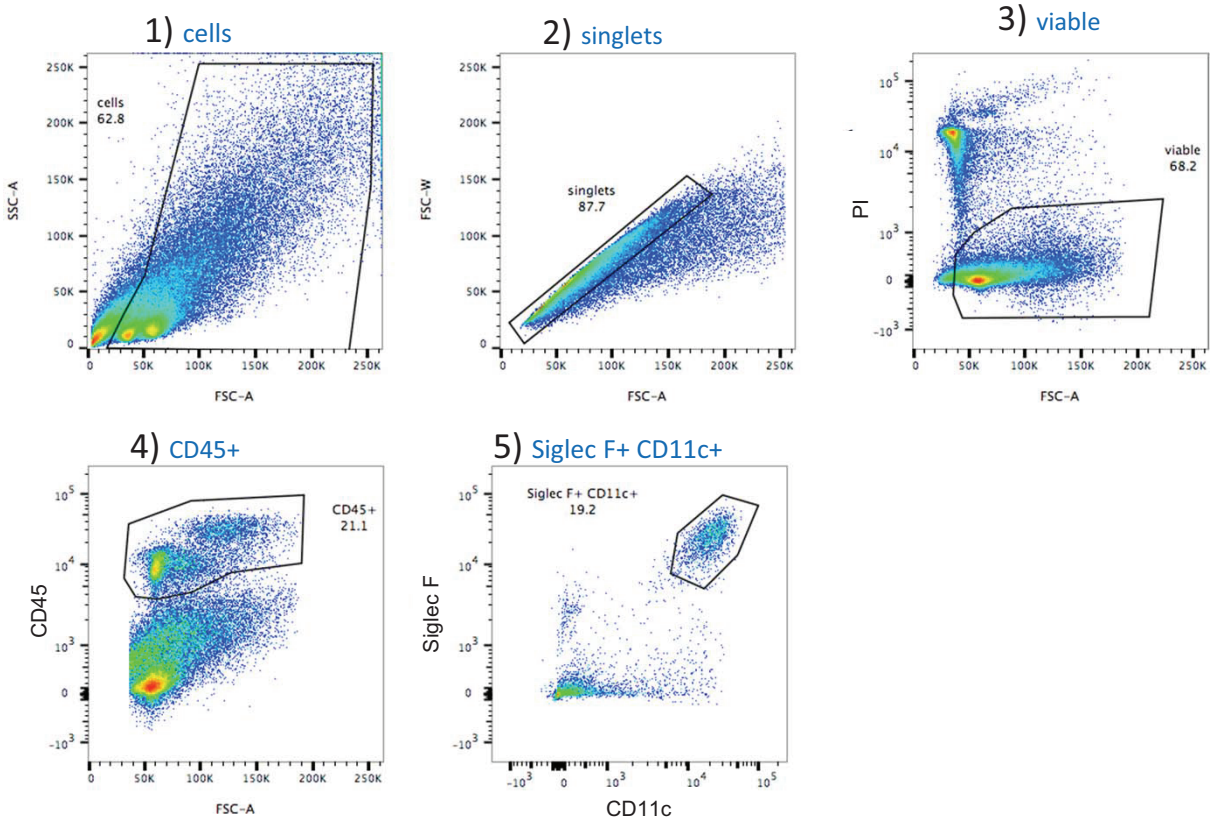
10000 cells
97.5% purity

Immunological Genome Project
ImmGen.org

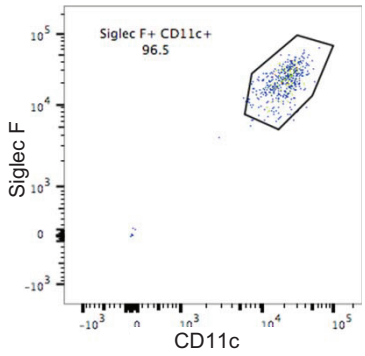
[Barbara Maier]
Miriam Merad Lab
Mount Sinai School of Medicine

MF.pIC.Alv.Lu: Poly(I:C)-stimulated lung alveolar macrophages

Pre-sort



Post-sort



Tissue Preparation: Collagenase V digestion, dounce glass homogenizer

Time from Mouse to Trizol: 09:00am-12:00am

Antibody (Clone) - Fluorochrome:

- Siglec F – PE
- CD11c – PE/Cy7
- CD45 – BV510
- viability - PI

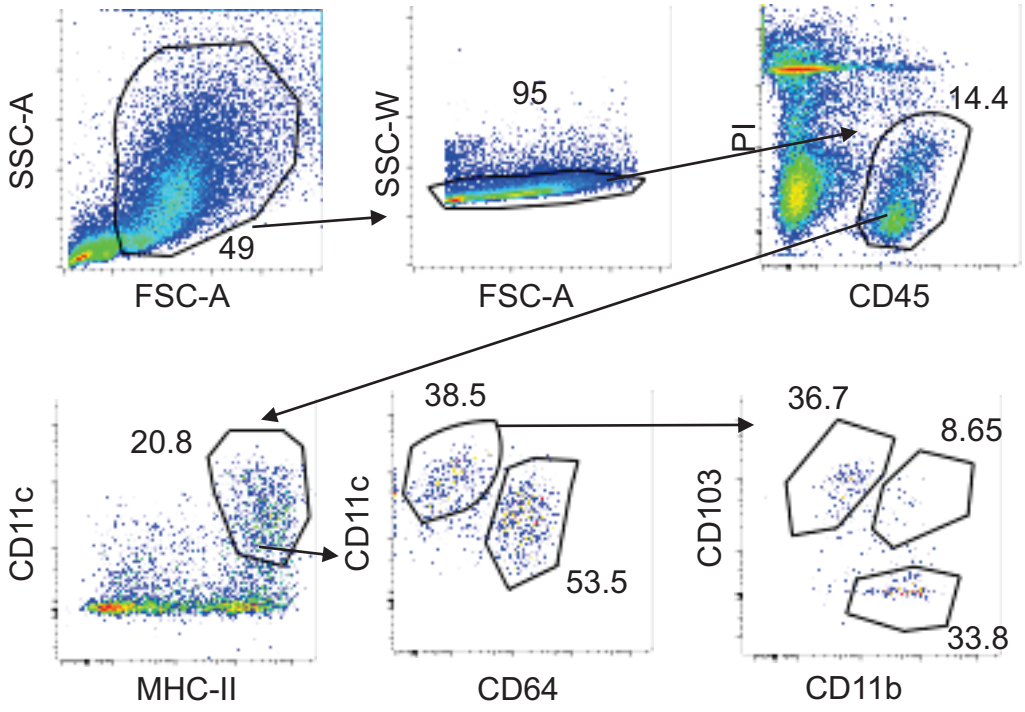
10000 cells
96.5% purity

Immunological Genome Project
ImmGen.org

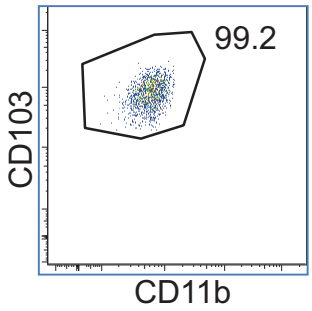
Merad Lab
Mount Sinai

DC.103+11b-.SI: Small intestine CD103+ CD11b- dendritic cells

Pre-sort



Second Sort



Tissue Preparation: Collagenase DNAse

Time from Mouse to Trizol: 9:00 am-2:00pm

Antibody (Clone) - Fluorochrome:

CD45 -- APC-Cy7
CD11c -- PE-Cy7
MHCII -- PacificBlue
CD64 -- APC
CD103 -- PE
CD11b -- FITC

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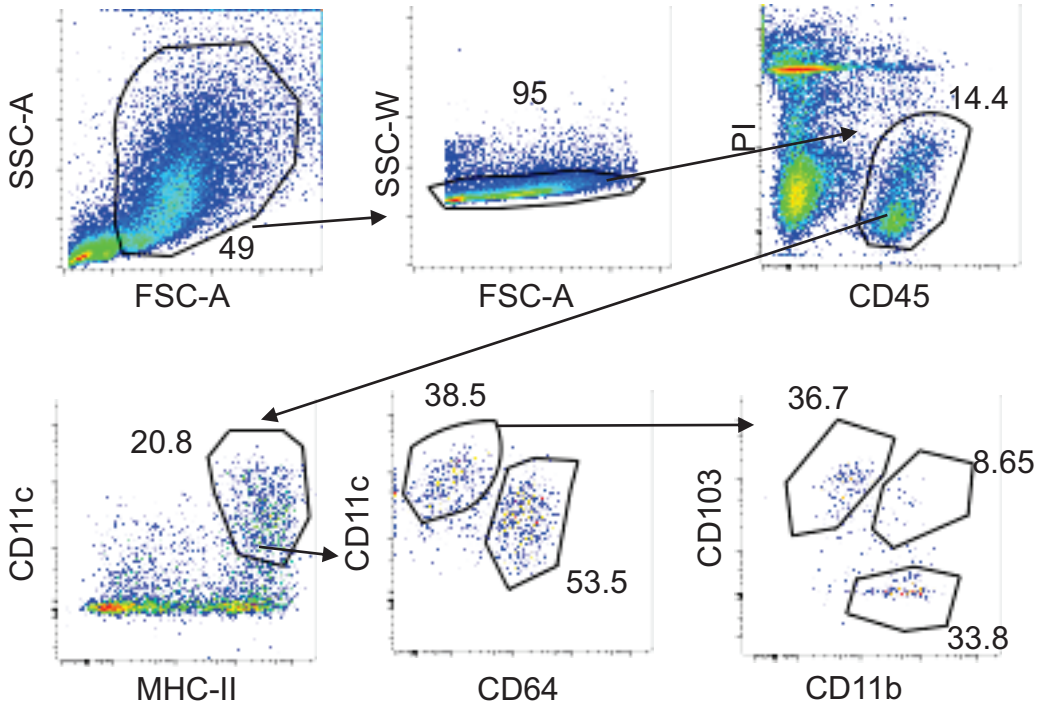
ImmGen.org

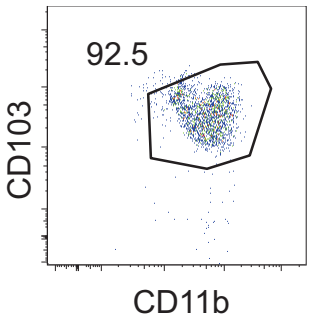
Merad Lab
Mount Sinai

DC.103+11b+.SI: Small intestine CD103+ CD11b+ dendritic cells

Pre-sort



Second Sort



Tissue Preparation: Collagenase DNAse

Time from Mouse to Trizol: 9:00 am-2:00pm

Antibody (Clone) - Fluorochrome:

CD45 -- APC-Cy7
CD11c -- PE-Cy7
MHCII -- PacificBlue
CD64 -- APC
CD103 -- PE
CD11b -- FITC

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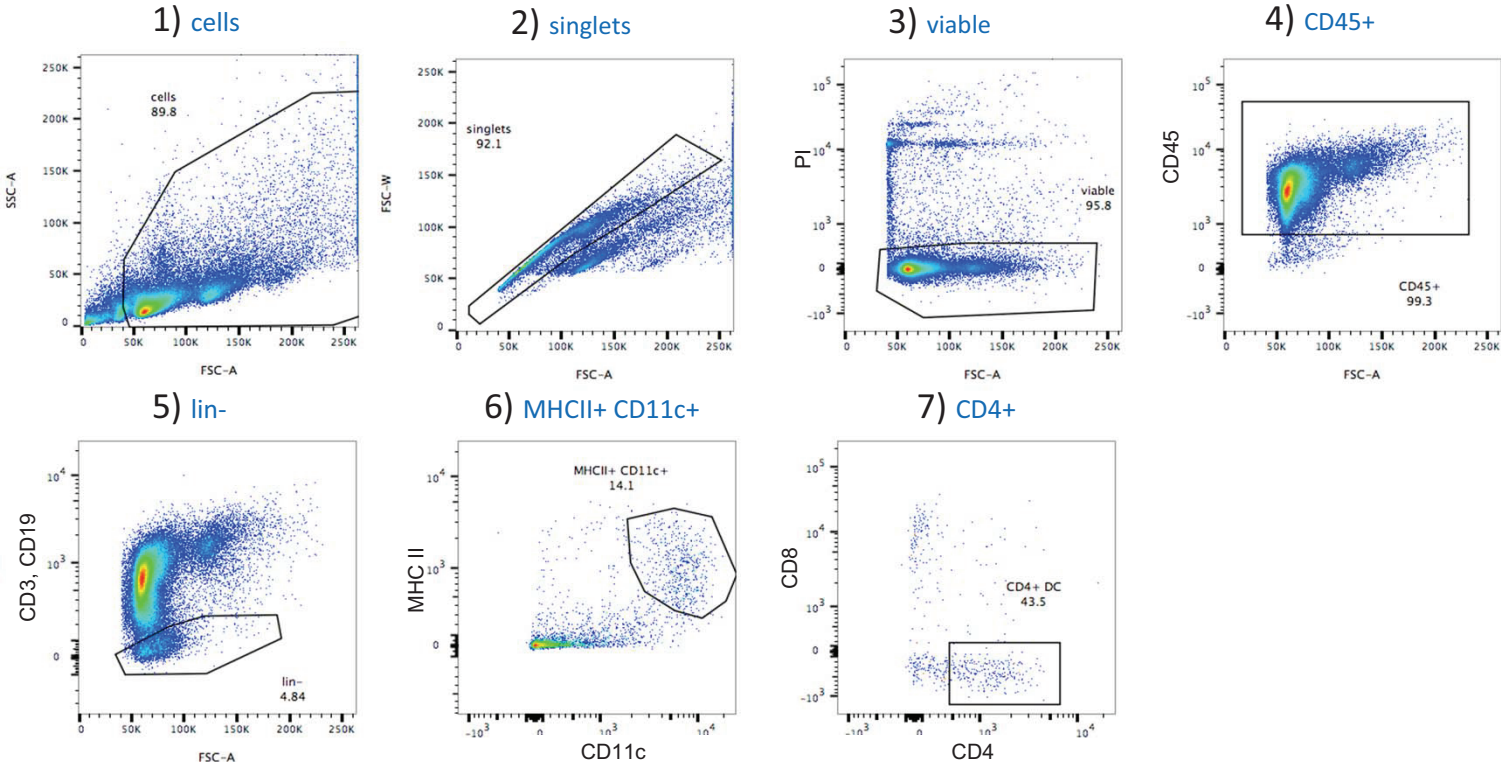
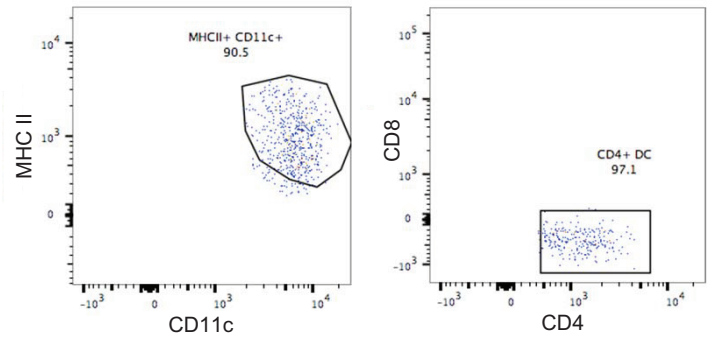
[Barbara Maier]
Miriam Merad Lab
Mount Sinai School of Medicine

DC.4+.Sp: Splenic CD4+ Dendritic cells

Pre-sort



Post-sort



Tissue Preparation: Collagenase V digestion, push through 70um cell strainer

Time from Mouse to Trizol: 09:00am-12:00pm

Antibody (Clone) - Fluorochrome:

- CD3, CD19 - FITC
- CD4 – APC
- CD11c – PE/Cy7
- CD8 – PE
- CD45 – BV510
- MHC II – APC/Cy7
- viability - PI

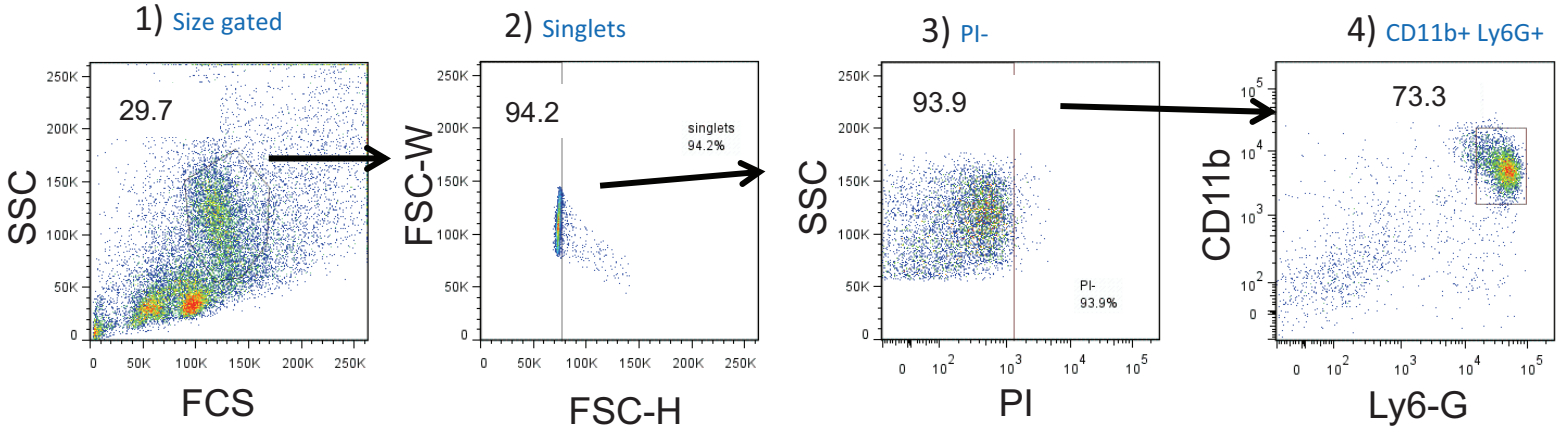
10000 cells
97.1% purity

Immunological Genome Project
ImmGen.org

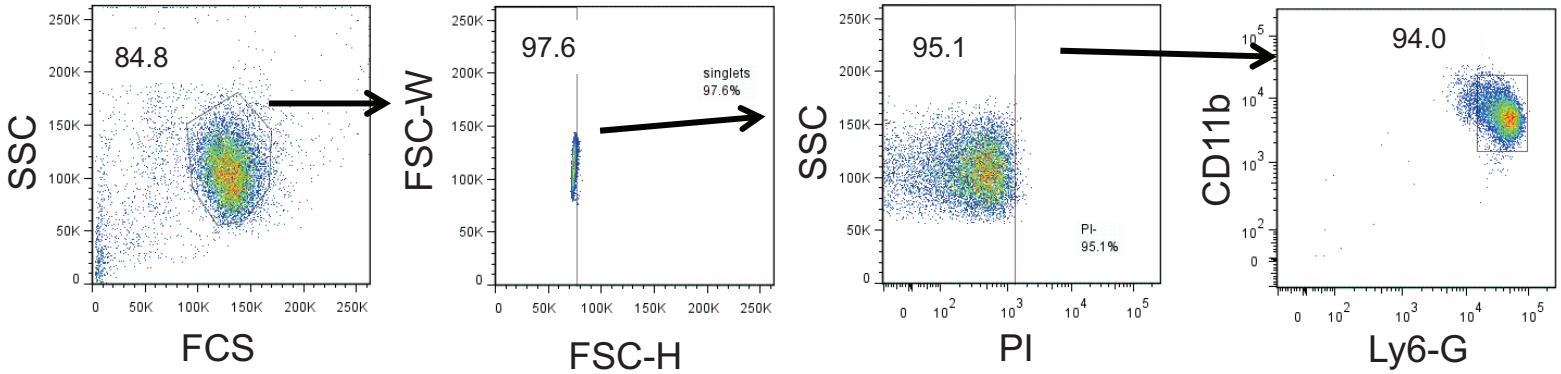
Monach & CBDM.Lab
Boston University School of Medicine

GN.BM: Bone marrow neutrophils

Pre-sort



2nd sorting



Tissue Preparation:
Flushed BM from femur using syringe, ACK lysis

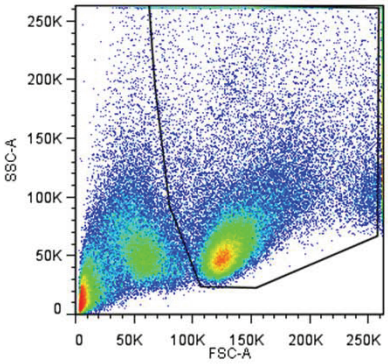
Time from Mouse to the 2nd sorting:
8am to 11am

Antibody (Clone) - Fluorochrome:
CD11b (M1/70) – PE
Ly6-G (RB6-8C5) – APC

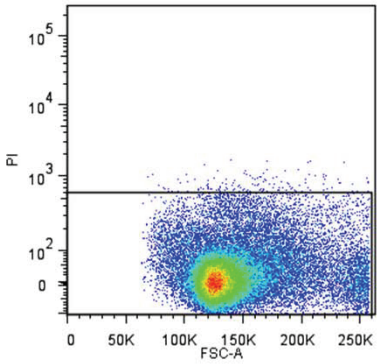
GN.SP: Splenic neutrophils

Pre-sort

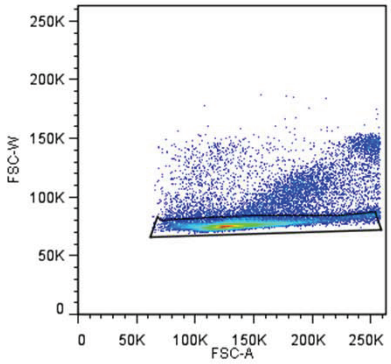
1) Cells



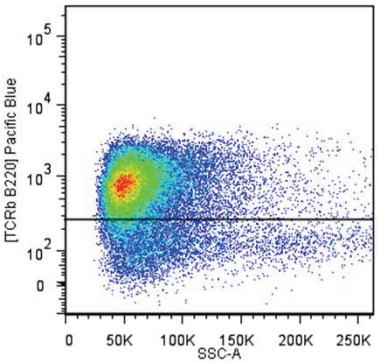
2) Live Cells



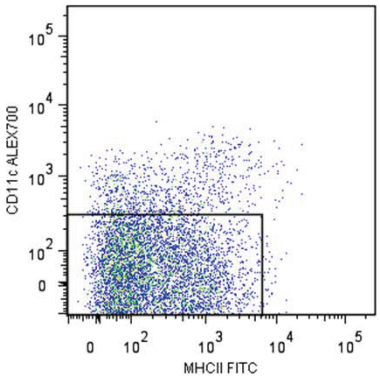
3) Singlets



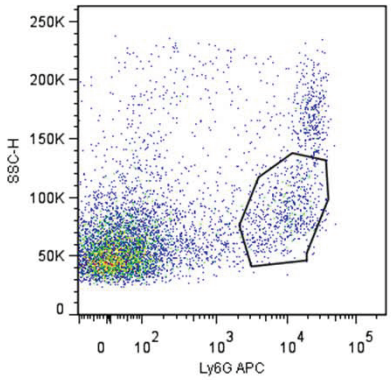
4) Dump: B220- and TCRb-



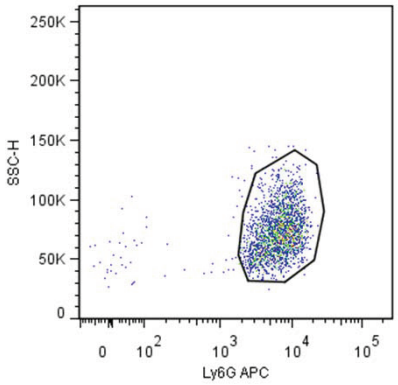
5) Gate out DCs



6) Neutrophils: Ly6G+



Post-sort



Tissue Preparation: Mechanical disruption of spleen
Time from Mouse to TCL Buffer: 8:30am-11:00am

Antibody (Clone) - Fluorochrome:

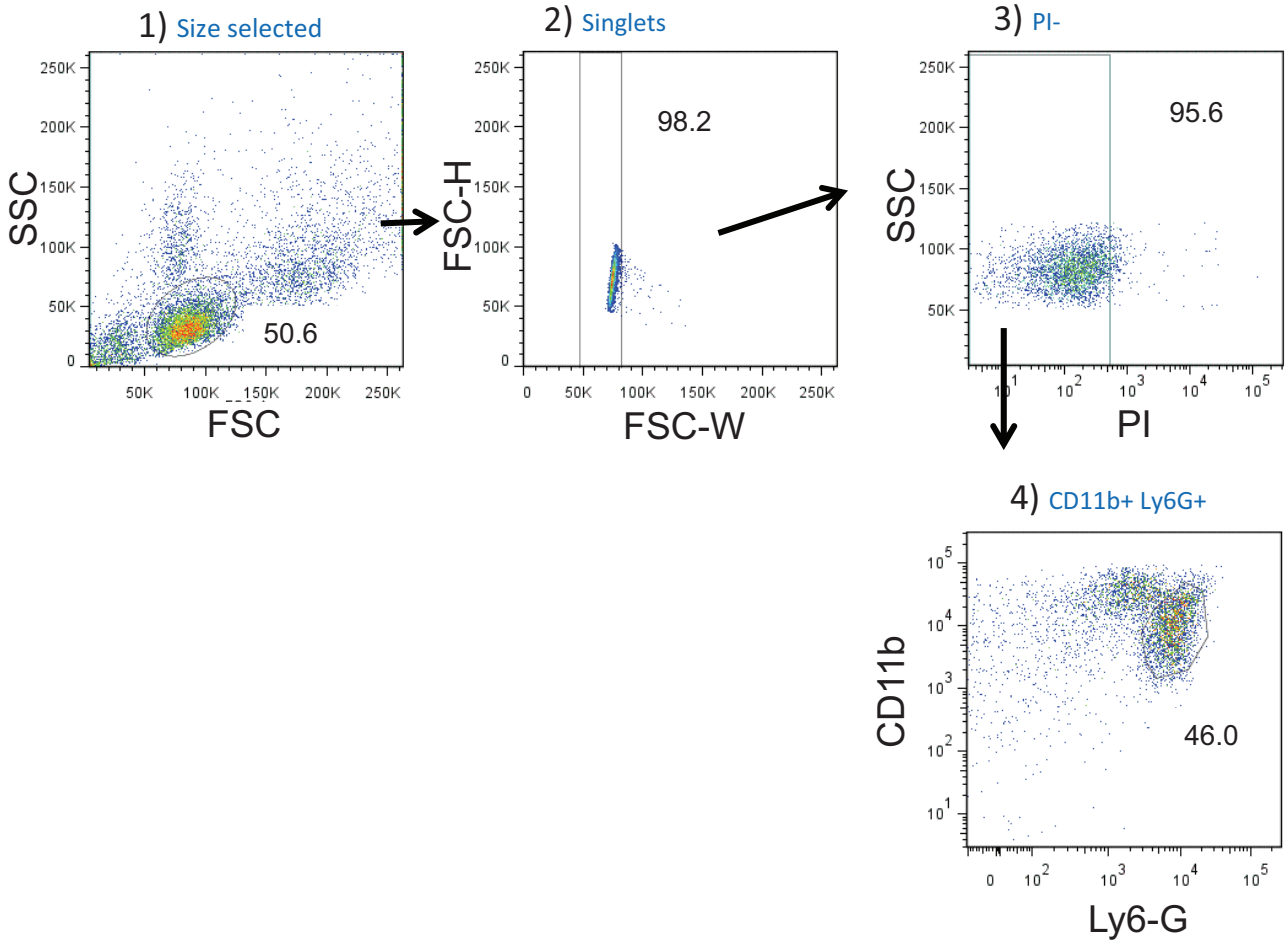
TCRb (H57-597)—Pacific Blue
B220 (RA3-6B2)—Pacific Blue
CD11c (N418)—Alexa 700
MHCII (M5/114.15.2)—FITC
Ly6G (RB6-8C5)—APC

Immunological Genome Project
ImmGen.org

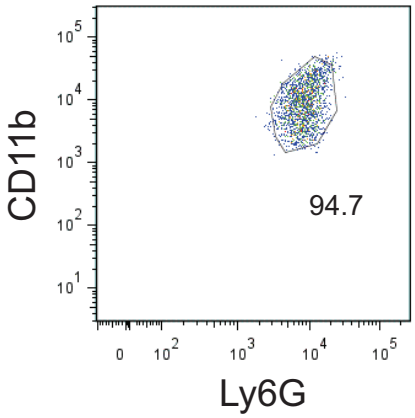
Monach & CBDM.Lab
Boston University School of Medicine

GN.Thio.PC: Thio-induced peritoneal cavity neutrophils

Pre-sort



2nd sorting



Tissue Preparation:
1ml 3% Thio(SIGMA, in dw) ip injected 18hrs before sac.
Peritoneal lavage with Phenol-red free DMEM, ACK lysis

Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
CD11b (M1/70) – PE
Ly6-G (RB6-8C5) – APC-Cy7

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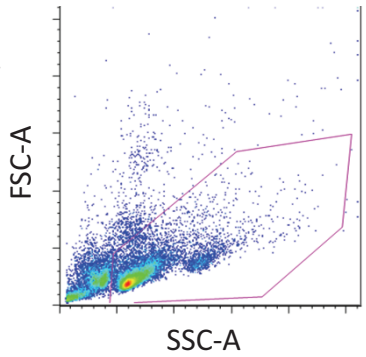
Tellier
NUTT LAB
WEHI

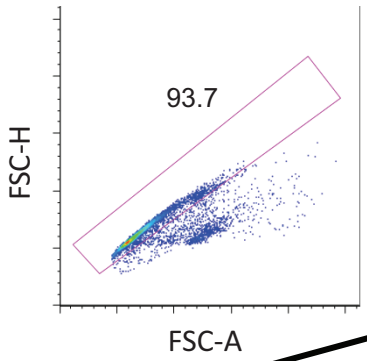
B.PB.Sp: Splenic plasmablasts

Pre-sort

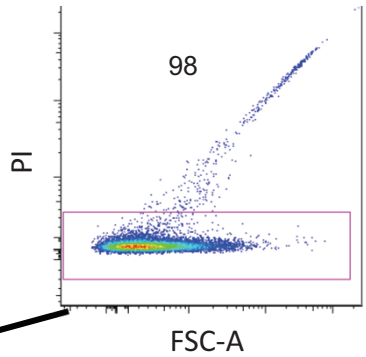
1) Lymphocytes



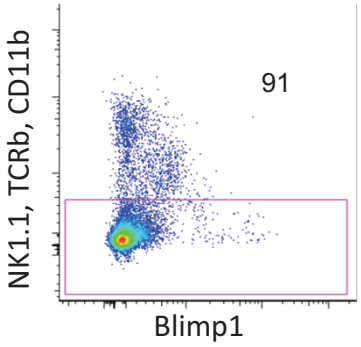
2) Singlets



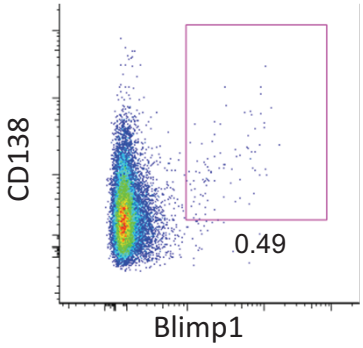
3) Live cells



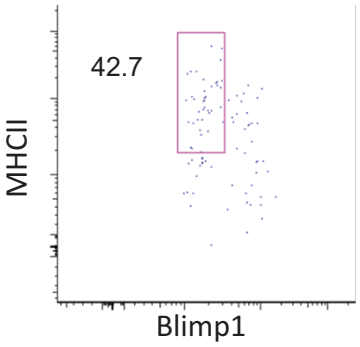
4) NK1.1- TCRb- CD11b-



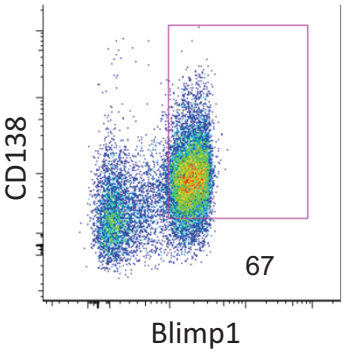
5) Antibody secreting cells

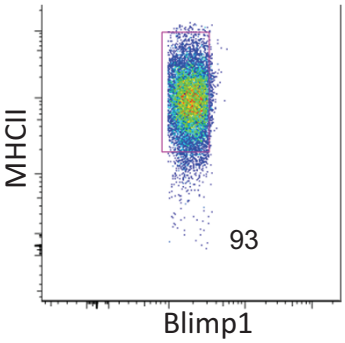


6) Plasmablasts



Second sort





Tissue Preparation: Bones crushed with mortar and pestle, ACK lysis of RBC
Time from Mouse to Trizol: 4 hours

Antibody (Clone) - Fluorochrome:
CD138 (281.2) – PE-Cy7
NK1.1 (PK136) – APC
CD11b (M1/70) – APC
TCRb (H57-597) – APC
MHCII ( ) – APC-Cy7

Over 90 %
before resort

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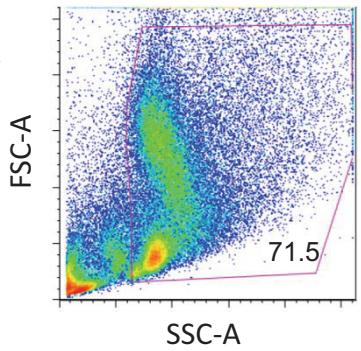
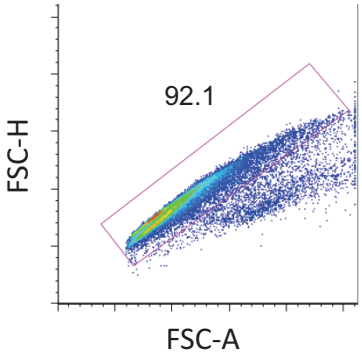
B.PC.BM: Bone marrow plasma cells

Pre-sort

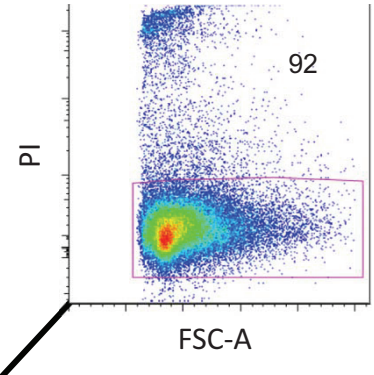
1) Lymphocytes



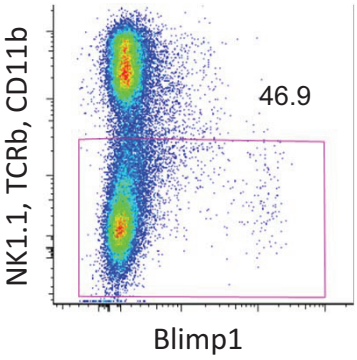
2) Singlets



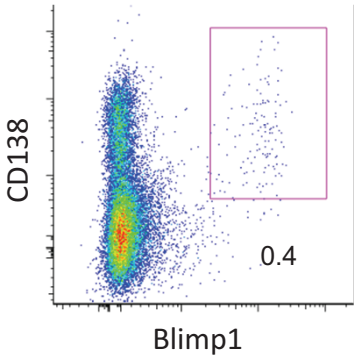
3) Live cells



4) NK1.1- TCRb- CD11b-



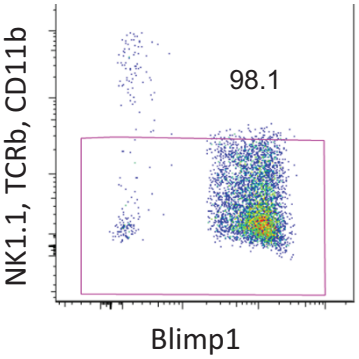
5) Plasma cells

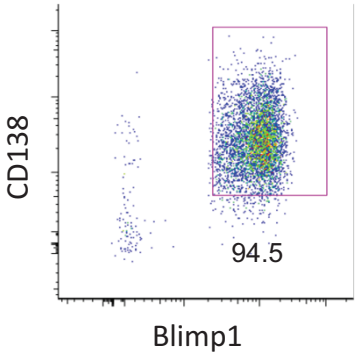


**alternatively, some people indicate what that panel is gated on – either is fine

---

Second sort





Tissue Preparation: Bones crushed with mortar and pestle, ACK lysis of RBC
Time from Mouse to Trizol: 4 hours

Antibody (Clone) - Fluorochrome:
CD138 (281.2) – PE-Cy7
NK1.1 (PK136) – APC
CD11b (M1/70) – APC
TCRb (H57-597) – APC

Over 90 %
before resort

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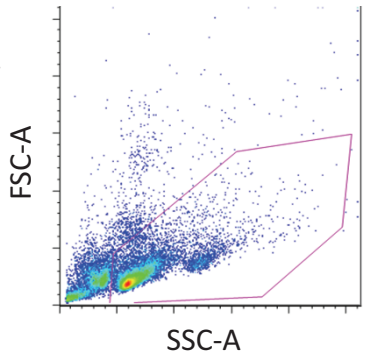
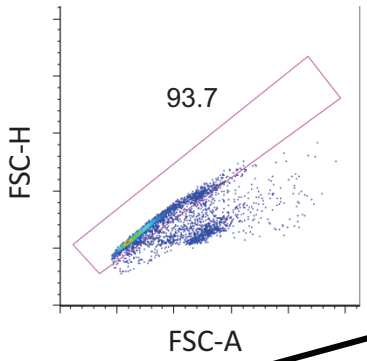
B.PC.Sp: Splenic plasma cells

Pre-sort

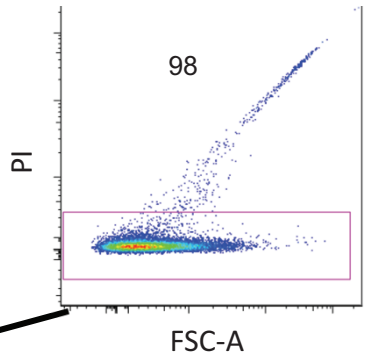
1) Lymphocytes



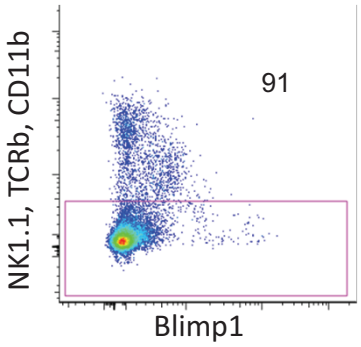
2) Singlets



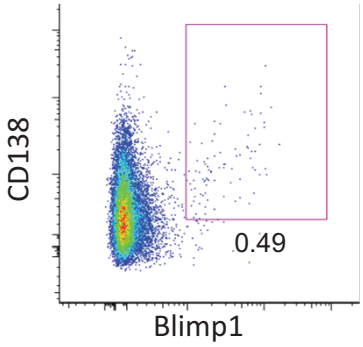
3) Live cells



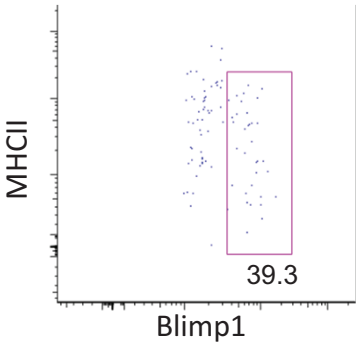
4) NK1.1- TCRb- CD11b-



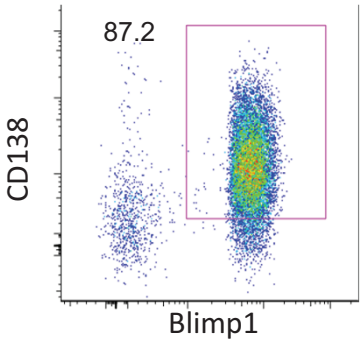
5) Antibody secreting cells

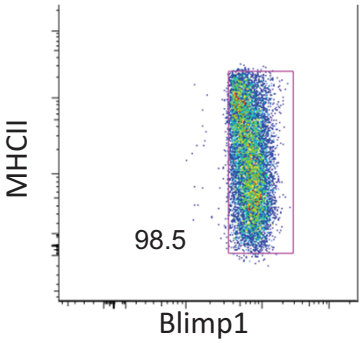


6) Plasma cells



Second sort





Tissue Preparation: Spleen homogenized through 100 µm cell-strainer, ACK lysis
Time from Mouse to Trizol: 4 hours

Antibody (Clone) - Fluorochrome:
CD138 (281.2) – PE-Cy7
NK1.1 (PK136) – APC
CD11b (M1/70) – APC
TCRb (H57-597) – APC
MHCII ( ) – APC-Cy7

Over 95 %
before resort

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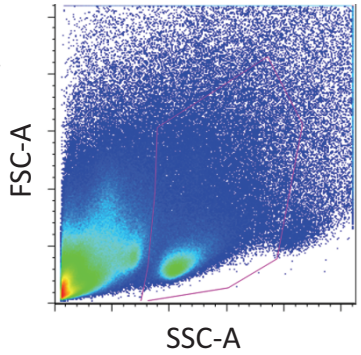
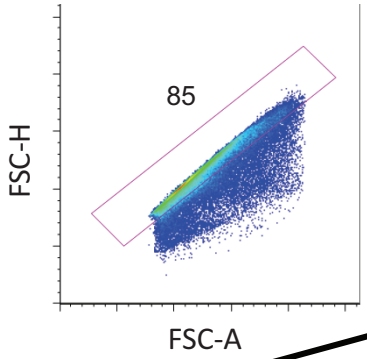
B.GC.CB.Sp: Splenic germinal center centroblasts

Pre-sort

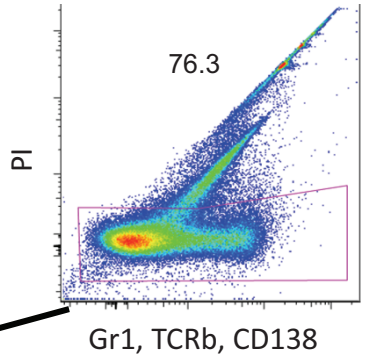
1) Lymphocytes



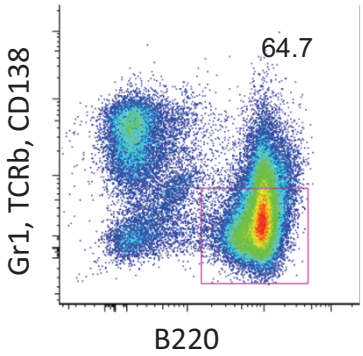
2) Singlets



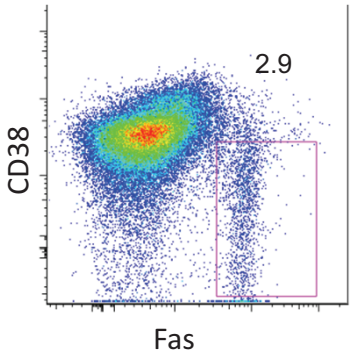
3) Live cells



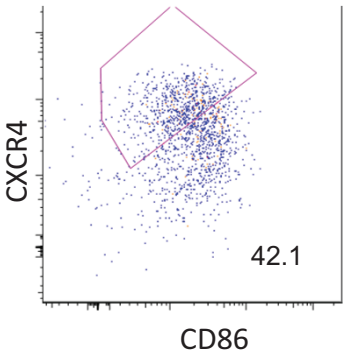
4) B cells



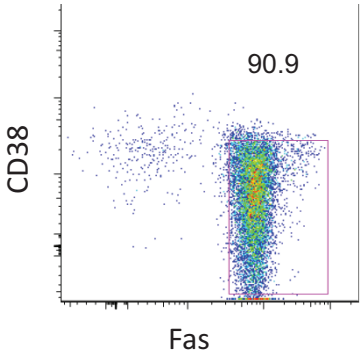
5) Germinal center

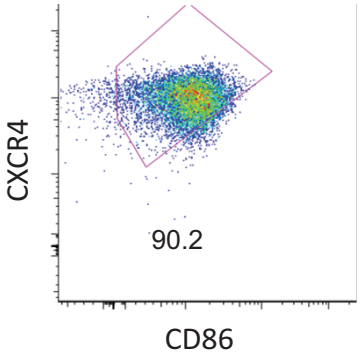


6) Centroblasts



Second sort





Tissue Preparation: Spleen homogenized through 100 µm cell-strainer, ACK lysis, positive selection with anti-IgG1-microbeads (Miltenyi)

Time from Mouse to Trizol: 5 hours

Antibody (Clone) - Fluorochrome:

CD138 (281.2) – PE
Gr1 (RB6-6C5) – PE
TCRb (H57-597) – PE
B220 (RA3-6B2) – FITC
Fas (Jo2) – PE-Cy7
CD38 (90) – AF780
CXCR4 (2B11) – BV421
CD86 (GL1) – BV605

Over 90 %
before resort

Immunological Genome Project
ImmGen.org

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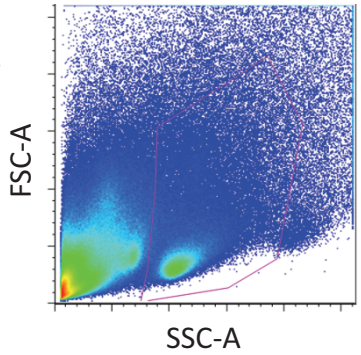
NUTT LAB
WEHI

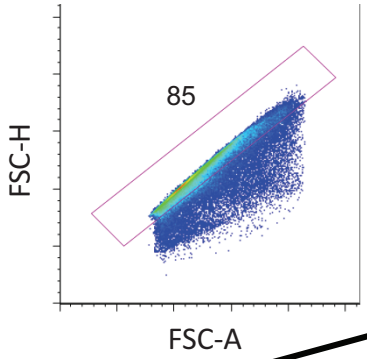
B.GC.CC.Sp: Splenic germinal center centrocytes

Pre-sort

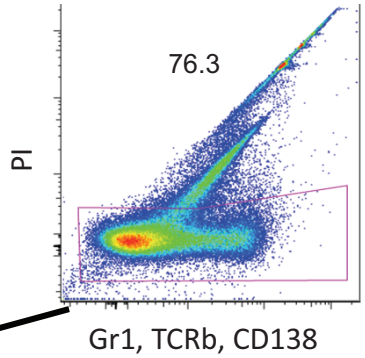
1) Lymphocytes



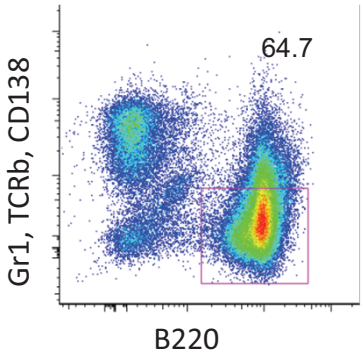
2) Singlets



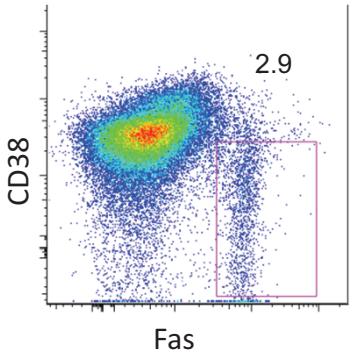
3) Live cells



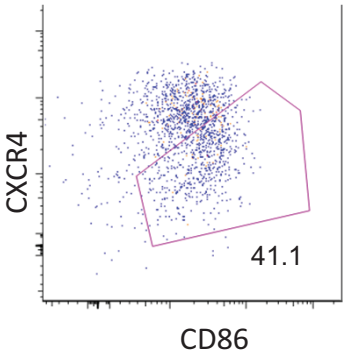
4) B cells



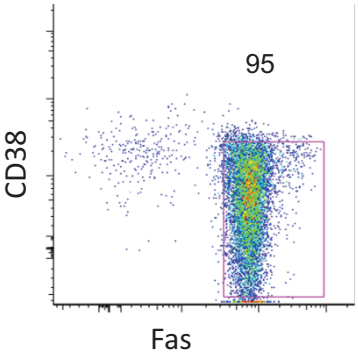
5) Germinal center

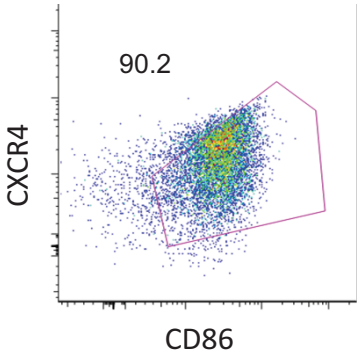


6) Centrocytes



Second sort





Tissue Preparation: Spleen homogenized through 100 µm cell-strainer, ACK lysis, positive selection with anti-IgG1-microbeads (Miltenyi)

Time from Mouse to Trizol: 5 hours

Antibody (Clone) - Fluorochrome:
CD138 (281.2) – PE
Gr1 (RB6-6C5) – PE
TCRb (H57-597) – PE
B220 (RA3-6B2) – FITC
Fas (Jo2) – PE-Cy7
CD38 (90) – AF780
CXCR4 (2B11) – BV421
CD86 (GL1) – BV605

Over 90 %
before resort

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ImmGen.org

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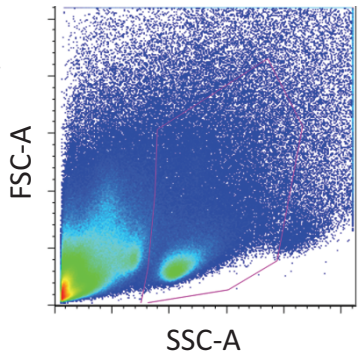
NUTT LAB
WEHI

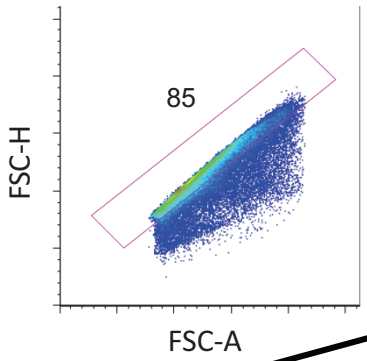
B.mem.Sp: Splenic memory B cells

Pre-sort

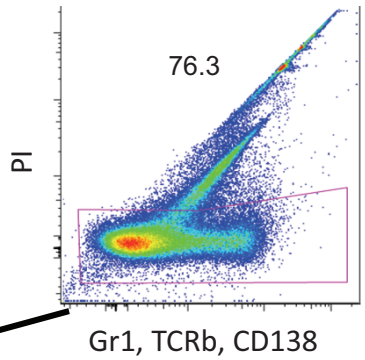
1) Lymphocytes



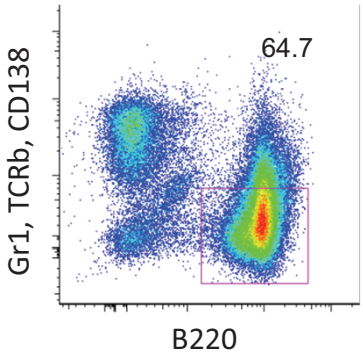
2) Singlets



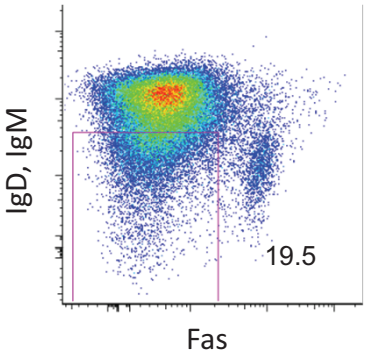
3) Live cells



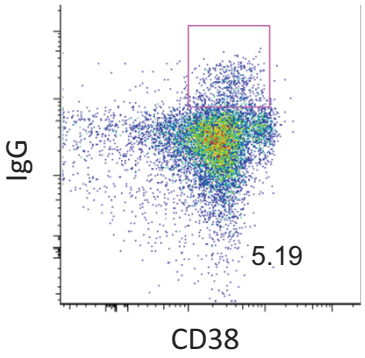
4) B cells



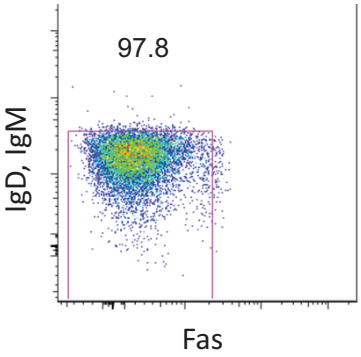
5) IgD-,IgM-, Fas-

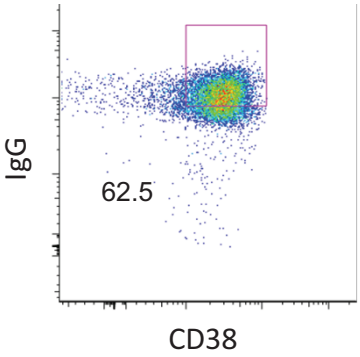


6) Memory B cells



Second sort





Tissue Preparation: Spleen homogenized through 100 µm cell-strainer, ACK lysis, positive selection with anti-IgG1-microbeads (Miltenyi)

Time from Mouse to Trizol: 5 hours

Antibody (Clone) - Fluorochrome:

CD138 (281.2) – PE
Gr1 (RB6-6C5) – PE
TCRb (H57-597) – PE
B220 (RA3-6B2) – FITC
Fas (Jo2) – PE-Cy7
CD38 (90) – AF780
IgM (II/41) – APC-eF780
IgD (11-26C) – APC-eF780
IgG (poly) – Cy5

Over 60 %
before resort

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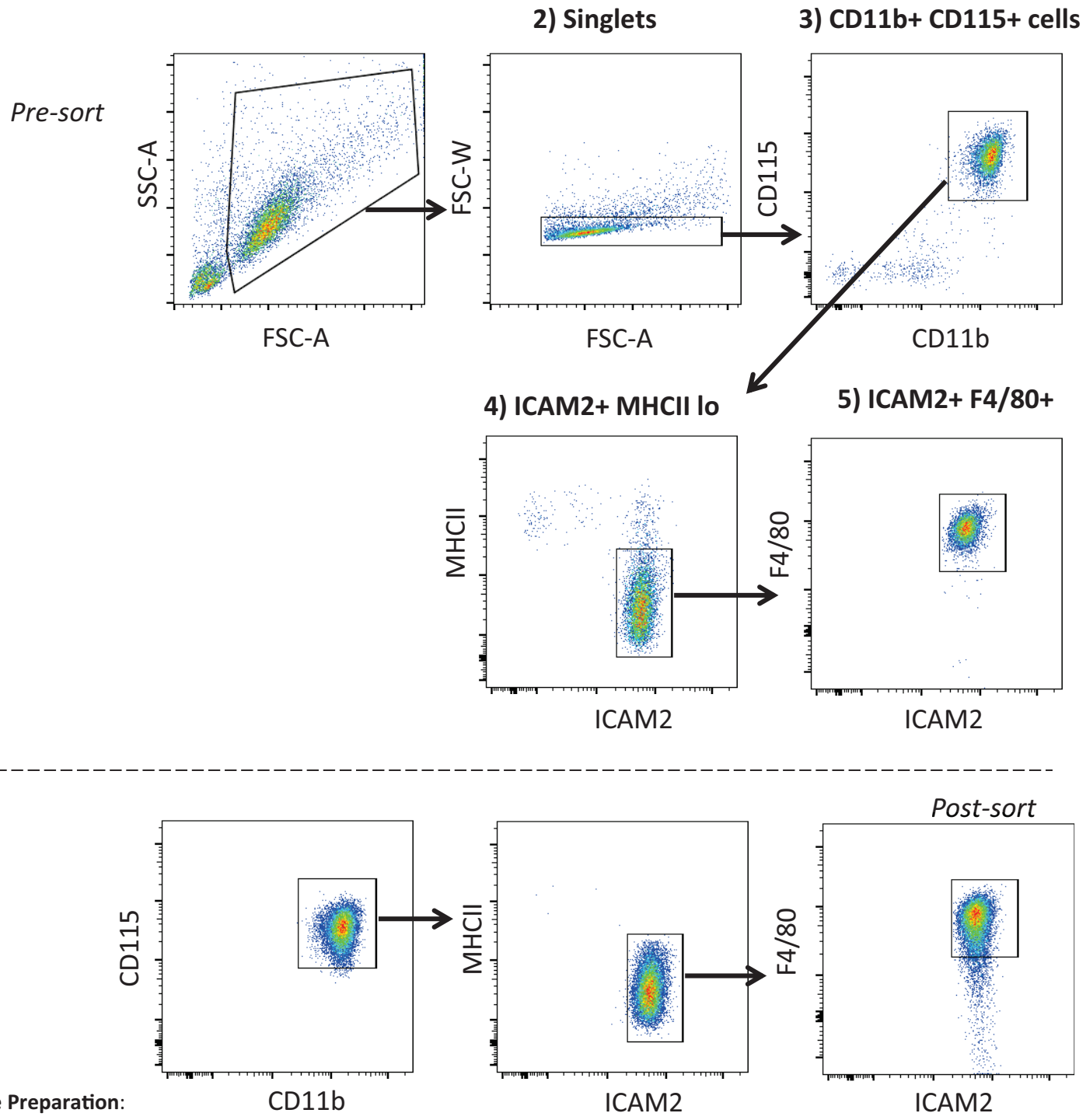
[Ki-Wook Kim] Randolph Lab.
Washington University St. Louis

MF.ICAM+480hi.PC: Large peritoneal cavity macrophages



Tissue Preparation:
Peritoneal lavage

Time from Mouse
30min antibody staining

Antibody (Clone) - Fluorochrome
ICAM2 -- Alexa 488
CD11b -- APC-Cy7
CD115 -- APC
MHCII -- efluor450
F4/80 -- PE-Cy7

10,000 cells
Purity >95%

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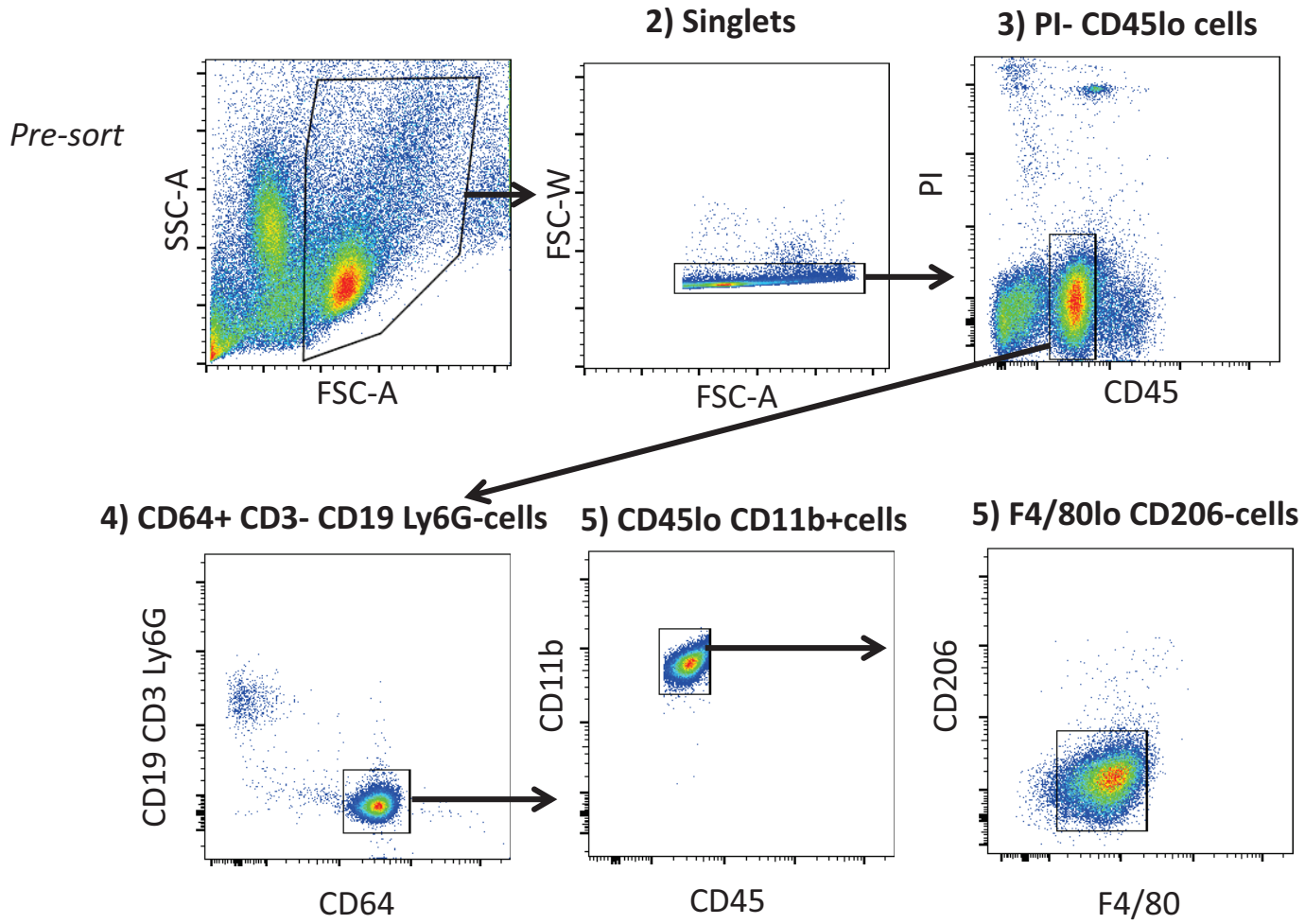
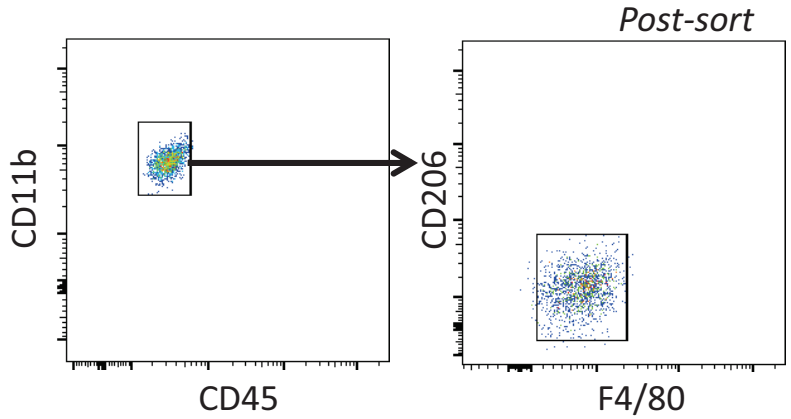
[Ki-Wook Kim] Randolph Lab.
Washington University St. Louis

MF.microglia.CNS: Microglias





Tissue Preparation:
Percoll

Time from Mouse
20min antibody staining

Antibody (Clone) - Fluorochrome
CD19, CD3, Ly6G -- PE
CD11b -- APC-Cy7
CD64 -- APC
CD45 -- efluor450
CD206 -- FITC
F4/80 -- PE-Cy7

10,000 cells
Purity >95%

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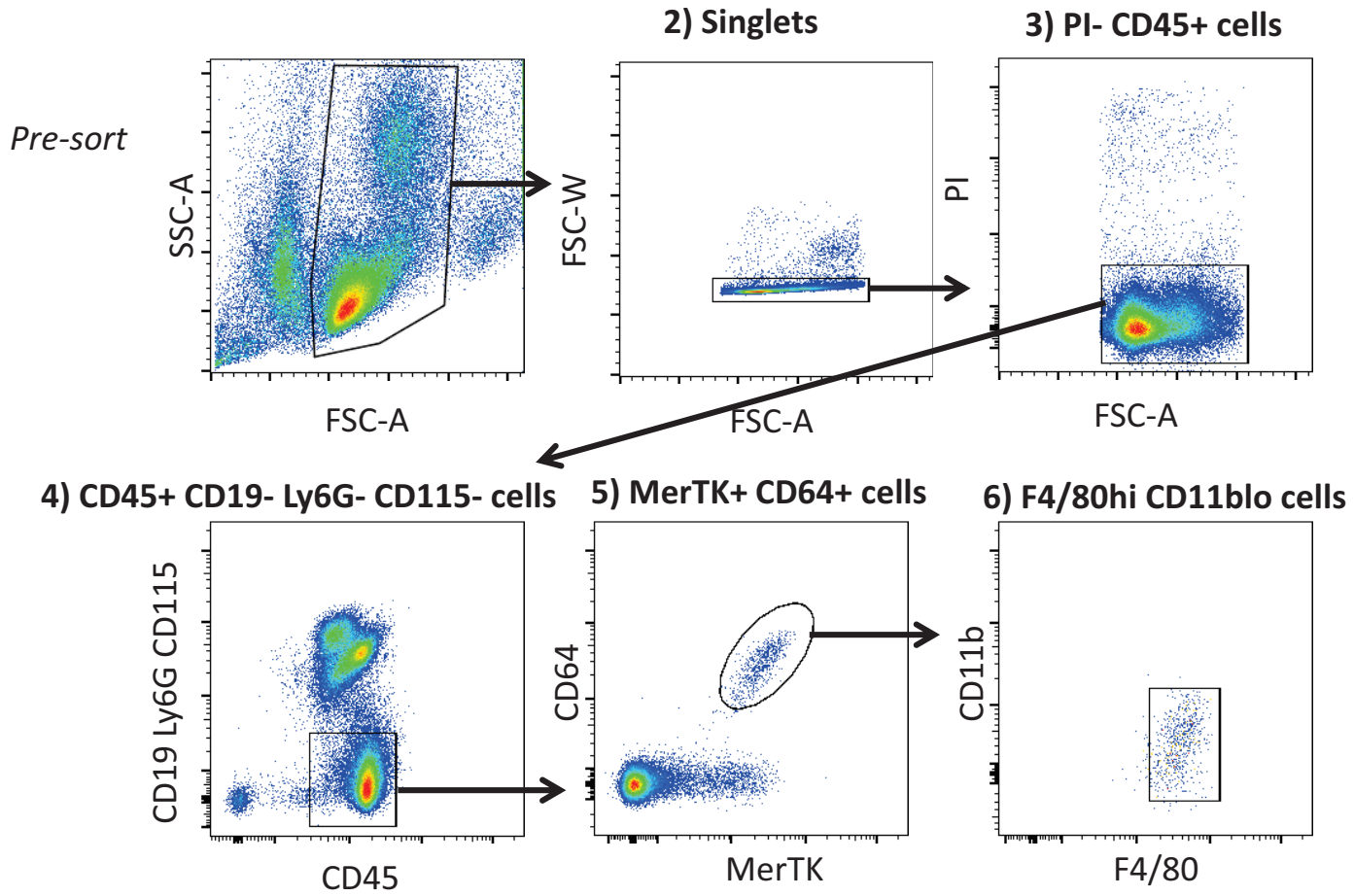
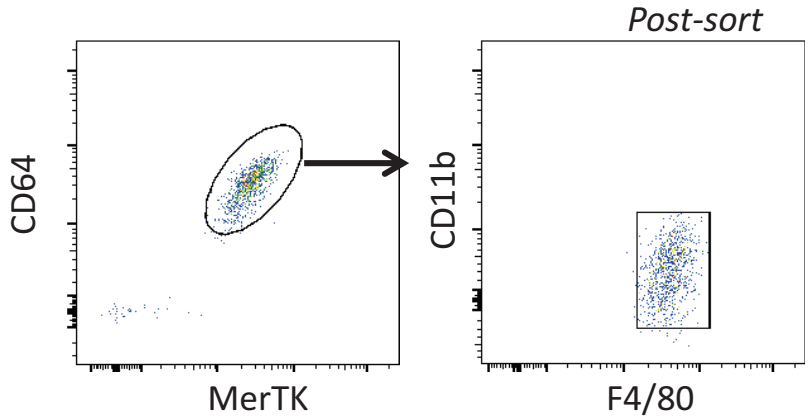
Washington University St. Louis

MF.RP.Sp: Spleen red pulp macrophages





Tissue Preparation:
Mechanical dissociation

Time from Mouse
30min antibody staining

Antibody (Clone) - Fluorochrome
CD19,Ly6G,CD115 -- PE
CD11b -- APC-Cy7
CD64 -- APC
CD45 -- efluor450
MerTK -- PE-Cy7
F4/80 -- Alexa488

10,000 cells
Purity >95%

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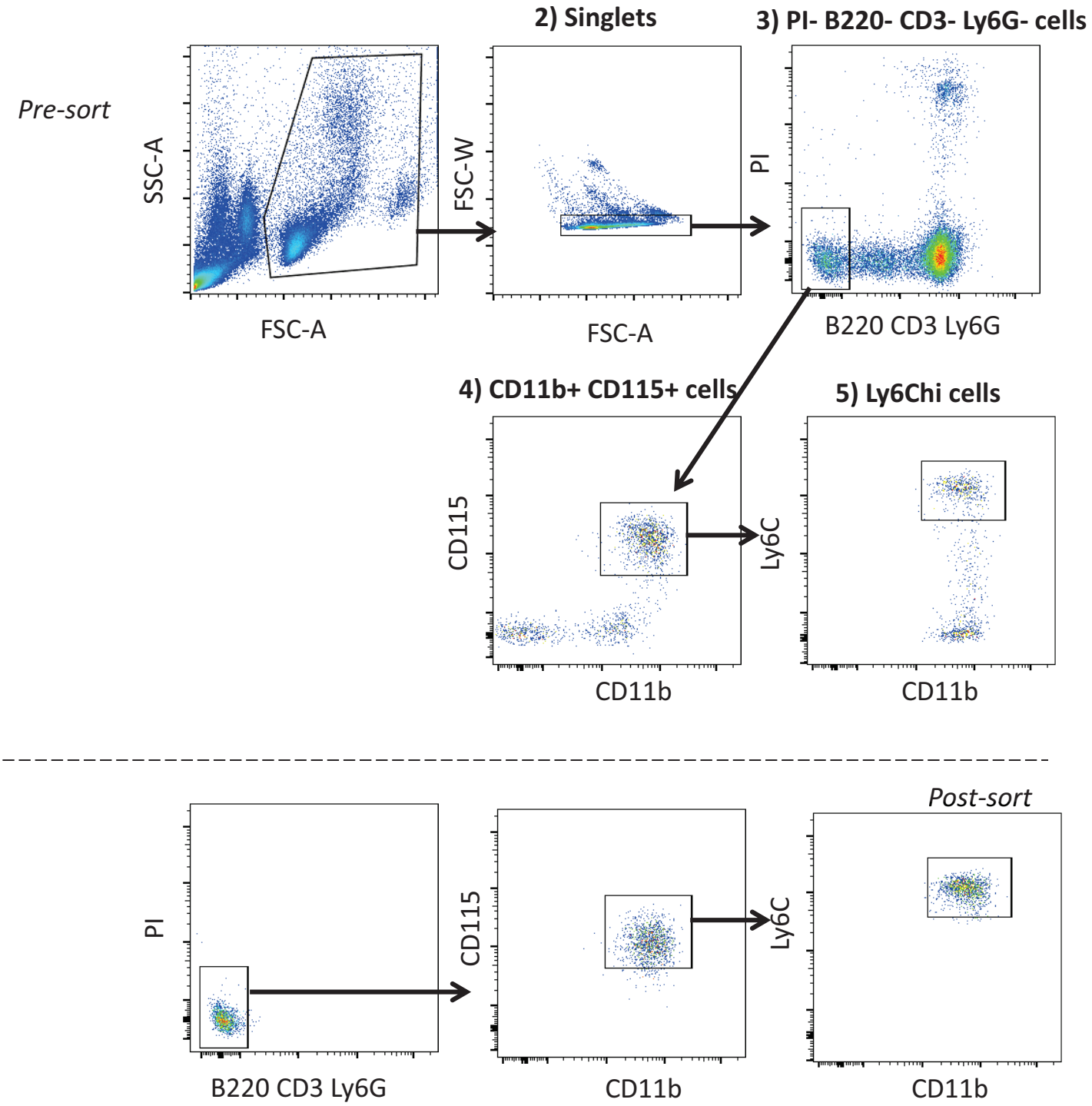
[Ki-Wook Kim] Randolph Lab.
Washington University St. Louis

Mo.6C+II-.Bl: Ly6Chi blood monocytes



Tissue Preparation:
500uL of Lysis buffer

Time from Mouse
20min antibody staining

Antibody (Clone) - Fluorochrome:
B220, CD3, Ly6G -- PE-Cy7
CD11b -- APC-Cy7
CD115 -- APC
Ly6C -- efluor450

10,000 cells
Purity>95%

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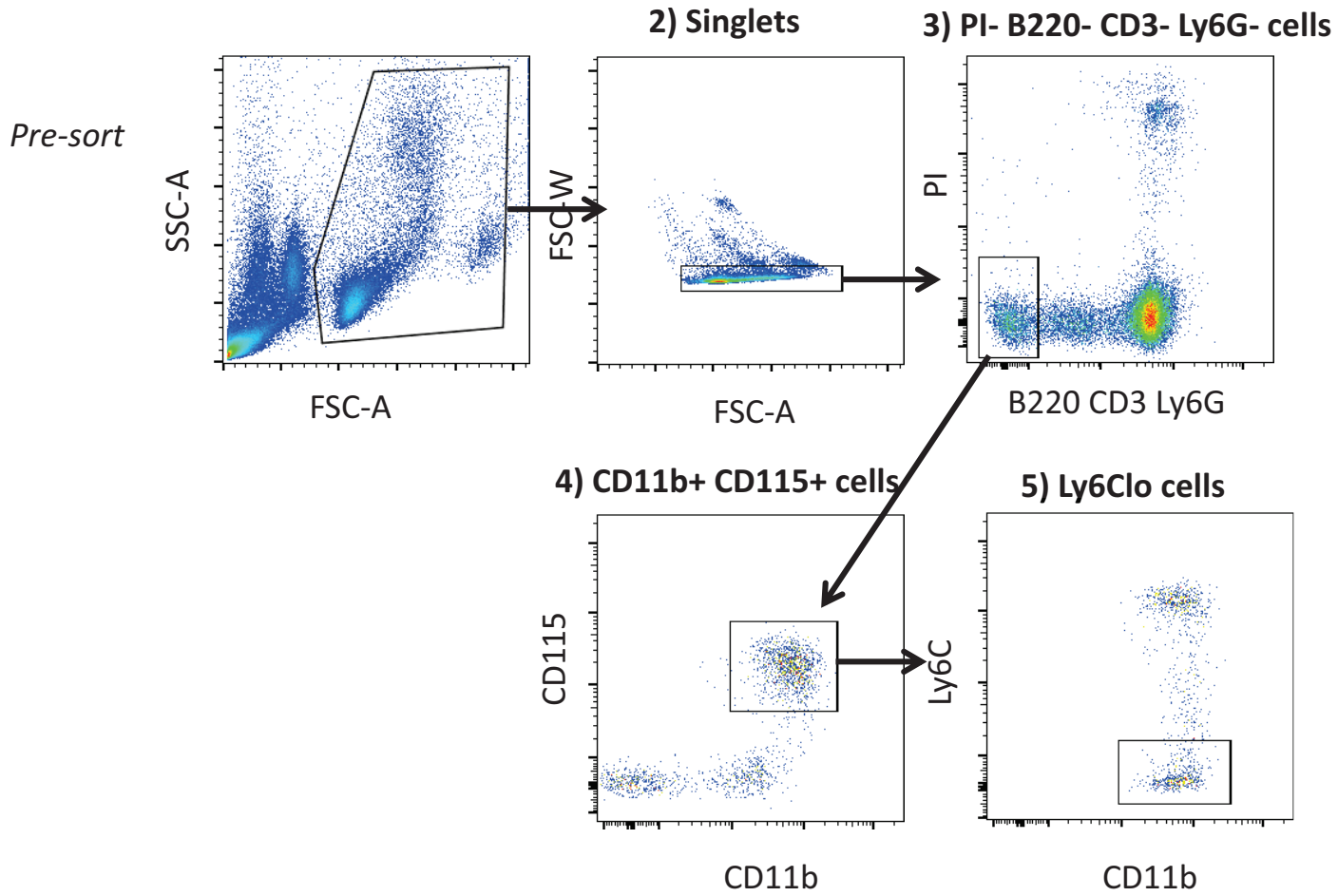
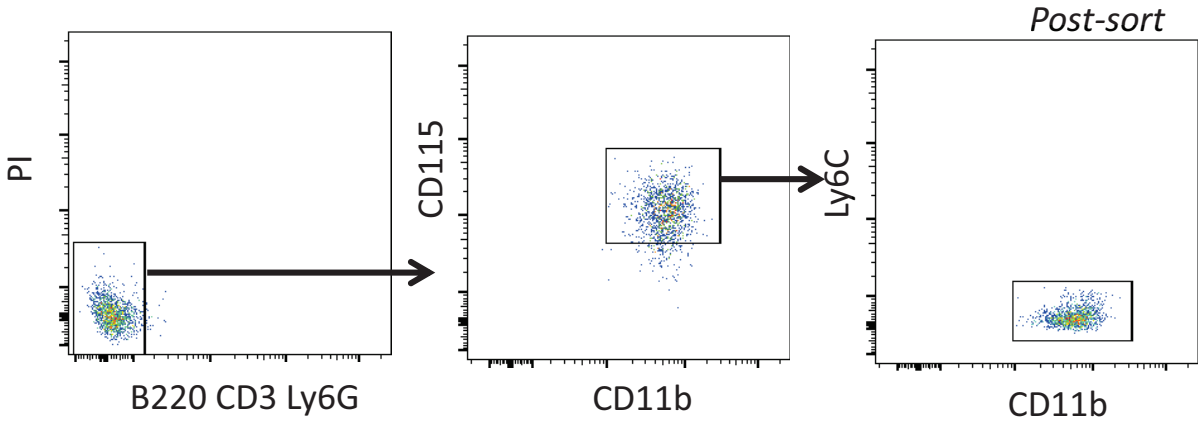
[Ki-Wook Kim] Randolph Lab.
Washington University St. Louis

Mo.6C-II-.Bl: Ly6Clo blood monocytes





Tissue Preparation:
500uL of Lysis buffer

Time from Mouse
20min antibody staining

Antibody (Clone) - Fluorochrome
B220, CD3, Ly6G -- PE-Cy7
CD11b -- APC-Cy7
CD115 -- APC
Ly6C -- efluor450

10,000 cells
Purity>95%

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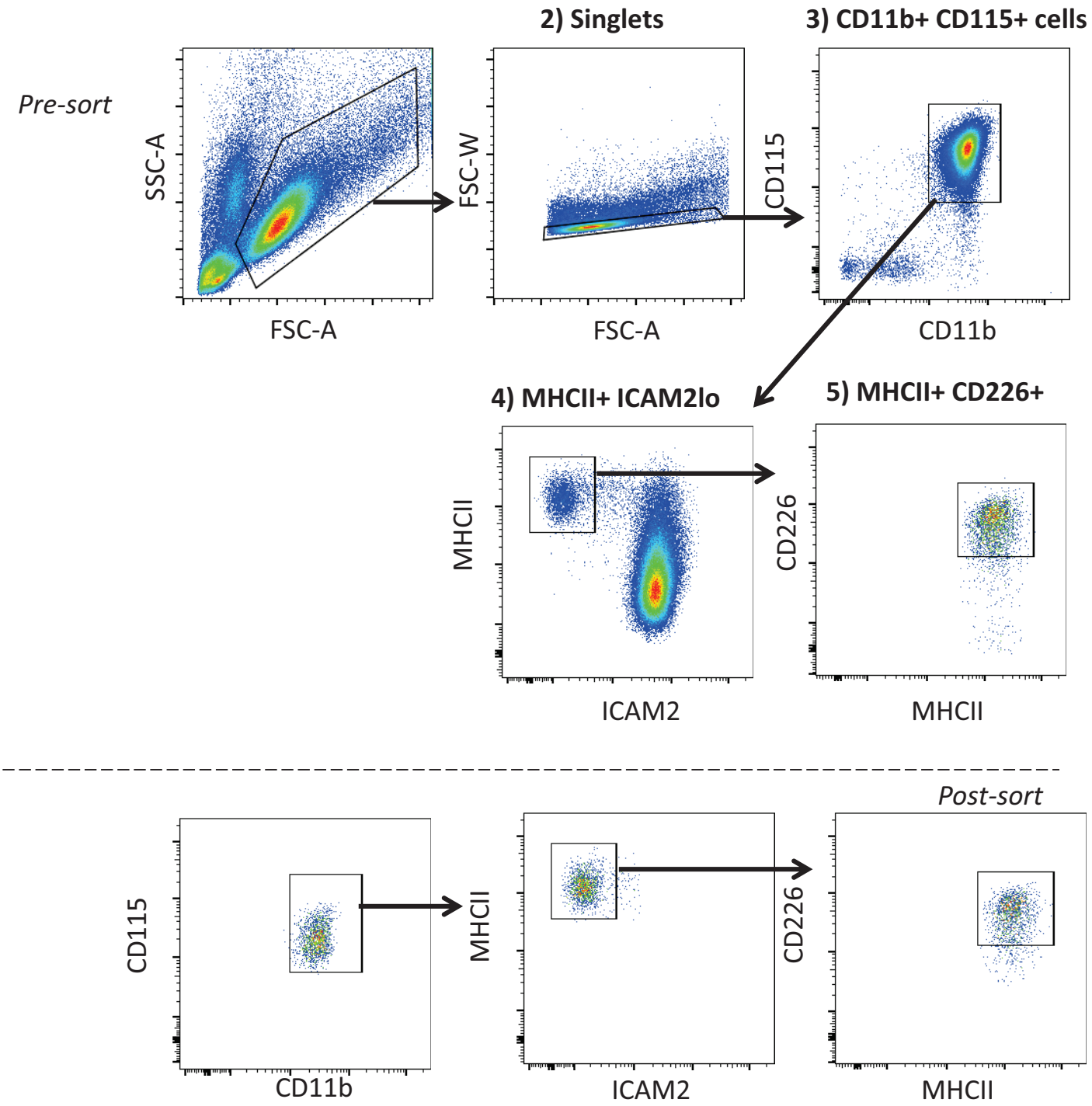
[Ki-Wook Kim] Randolph Lab.
Washington University St. Louis

MF.226+II+480lo.PC: Small peritoneal cavity macrophages



Tissue Preparation:
Peritoneal lavage

Time from Mouse
20min antibody staining

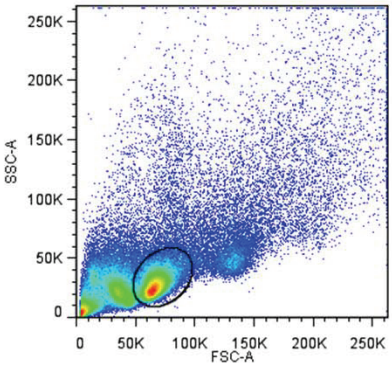
Antibody (Clone) - Fluorochrome
ICAM2 -- Alexa 488
CD11b -- APC-Cy7
CD115 -- APC
MHCII -- efluor450
CD226 -- PE

10,000 cells
Purity >95%

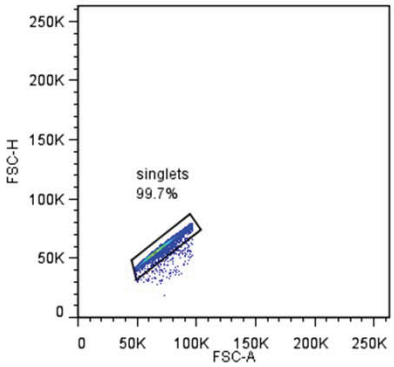
B.Fem.SP: Splenic B cell (Female)

Pre-sort

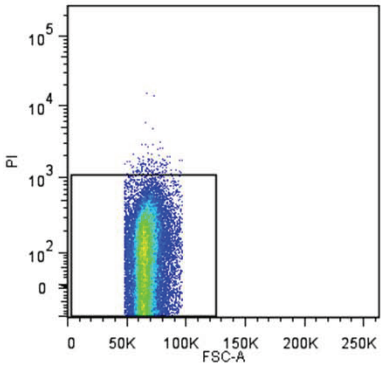
1) Lymphocytes



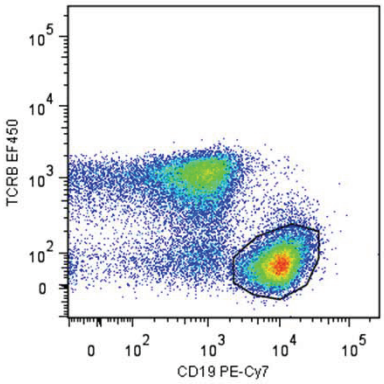
2) Singlets



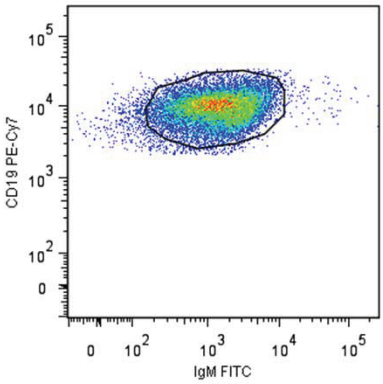
3) Live Cells



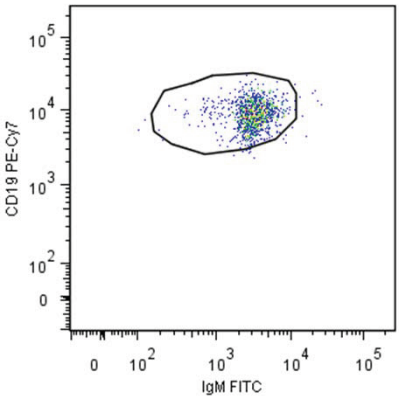
4) CD19+ Cells



6) B Cells



Post-sort



Tissue Preparation: Mechanical disruption of spleen
Time from Mouse to TCL Buffer: 10:30am-12:00pm

Antibody (Clone) - Fluorochrome:
TCRb (H57-597)—Pacific Blue
CD19 (1D3)—PE-Cy7
IgM (eB121-15F9)—FITC

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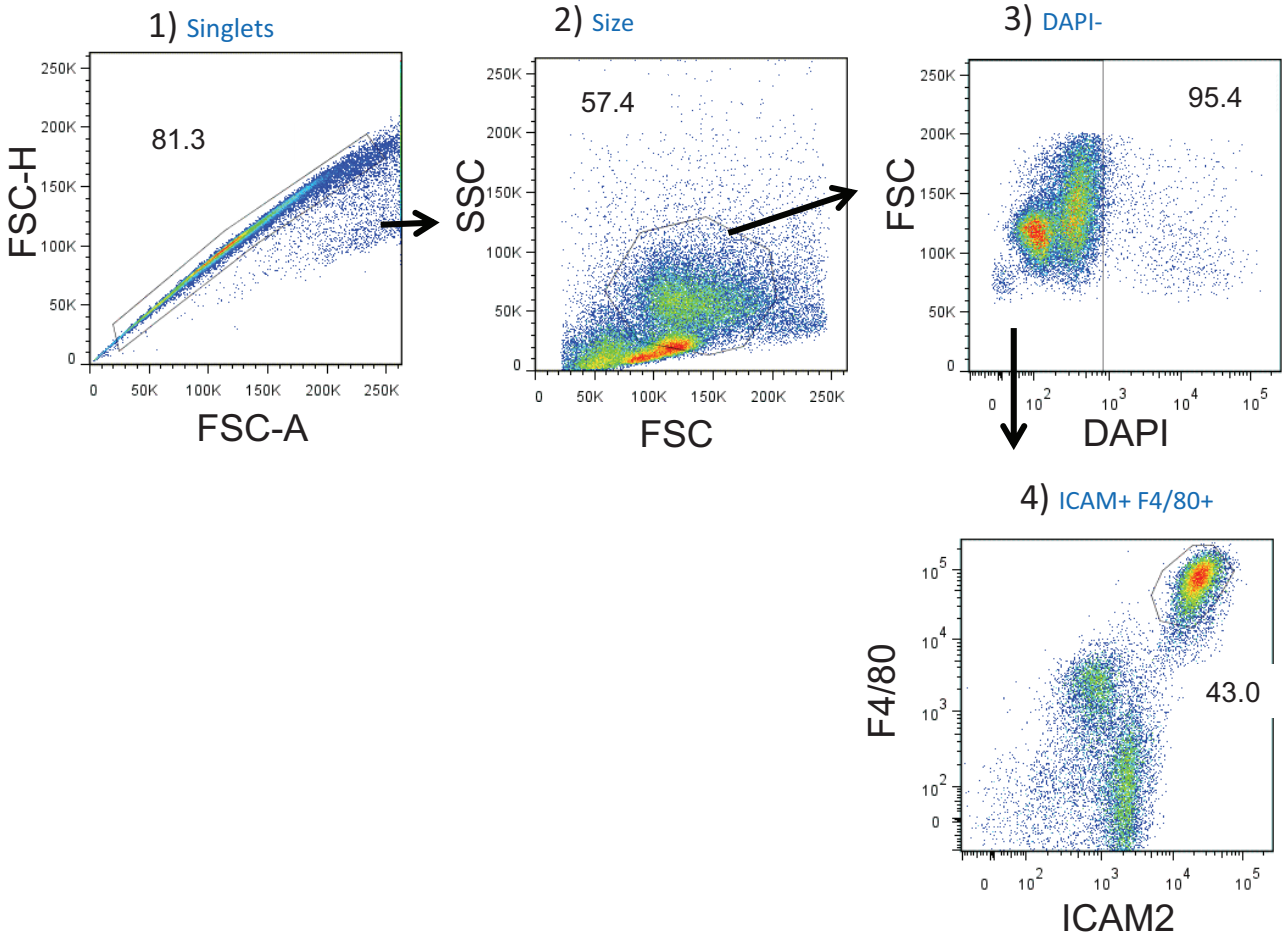
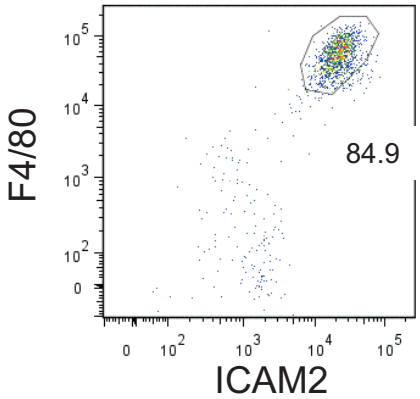
Shay & CBDM.Lab
Ben-Gurion University of the Negev

MF.Fem.PC: Peritoneal cavity macrophages (female)

Pre-sort



2nd sorting



Tissue Preparation:
Peritoneal lavage with Phenol-red free DMEM, ACK lysis

Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
F4/80 (BM8) -- PE-Cy7
ICAM2 (3C4 (mIC2/4)) -- FITC

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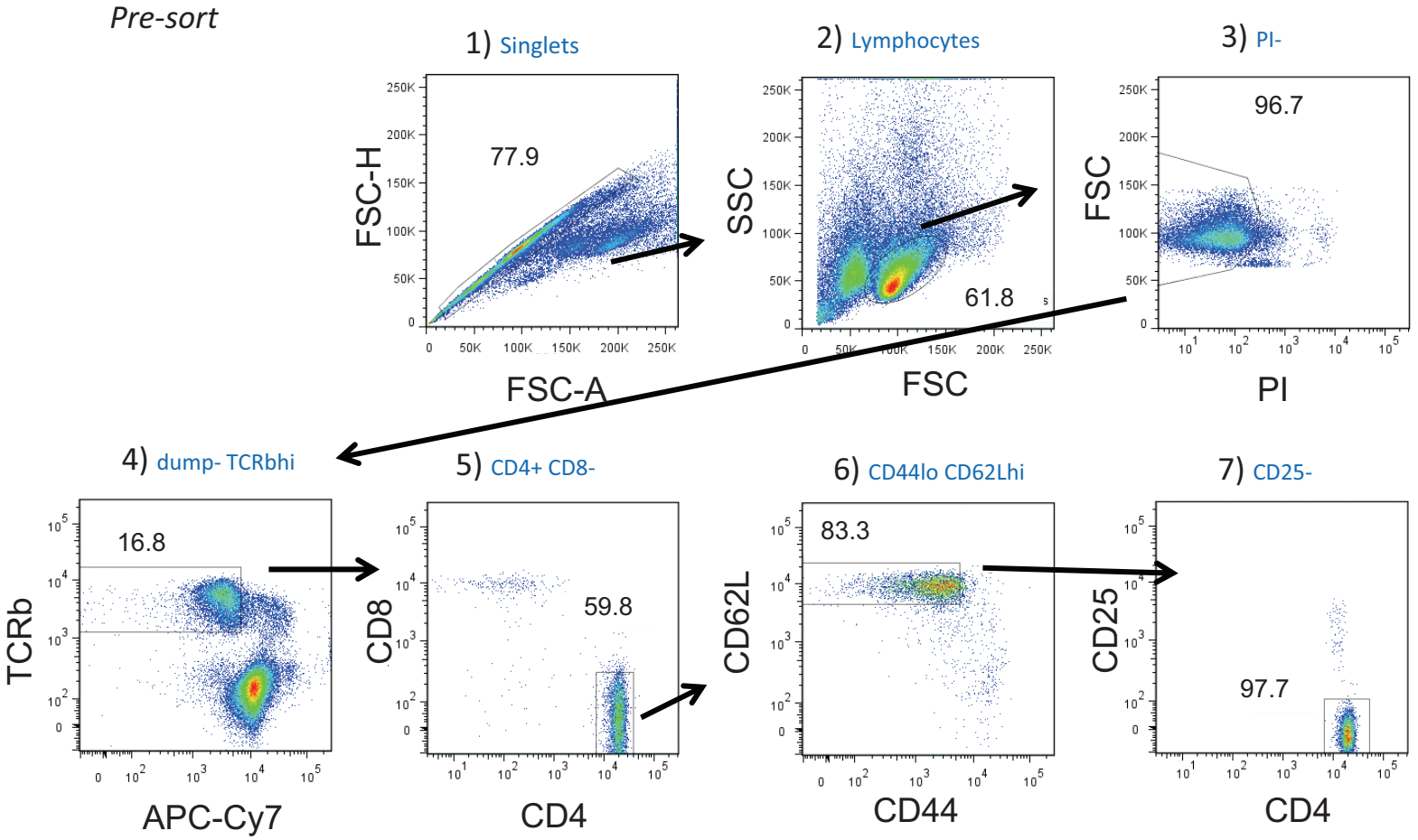
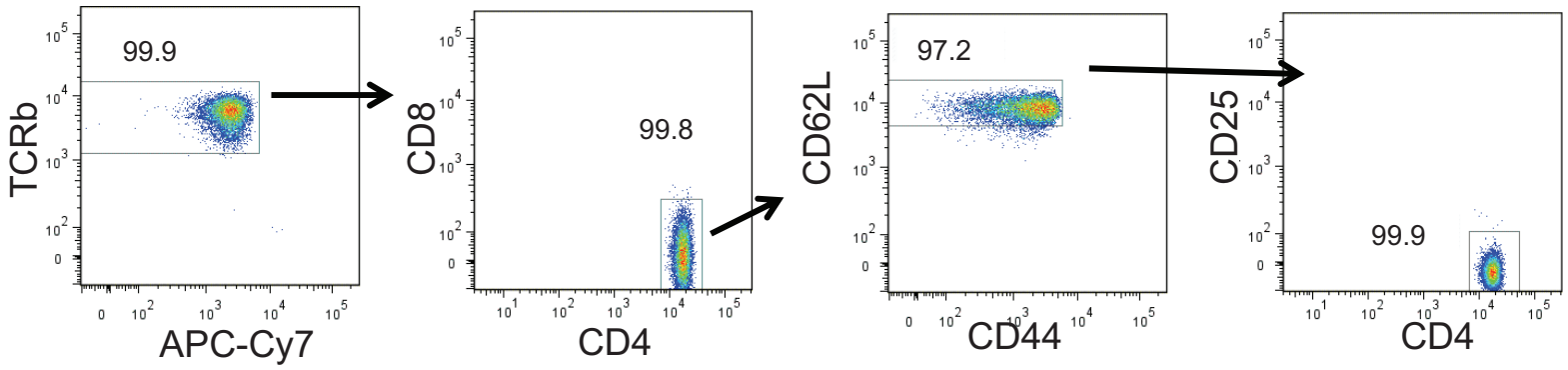
Shay & CBDM.Lab
Ben-Gurion University of the Negev

T.4.Nve.Fem.Sp: Splenic Naïve CD4 T cells (female)

Pre-sort



2nd sorting



Tissue Preparation:
100um mesh/syringe plunger teasing, ACK lysis

Time from Mouse to the 2nd sorting:
8am to 10am

Antibody (Clone) - Fluorochrome:
CD4 (RM4-5) – PE, CD8a (53-6.7) – A700, TCRb (H57-597) – eF450,
CD44(IM7) – FITC, CD62L(Mel-14) – PE-Cy7, CD25(PC61) -- APC
dump = CD19(1D3), Ter119(Ter-119) , Gr1(RB6-8C5) – APC-eF780
NK1.1(PK136), F4/80(BM8) -- APC-Cy7

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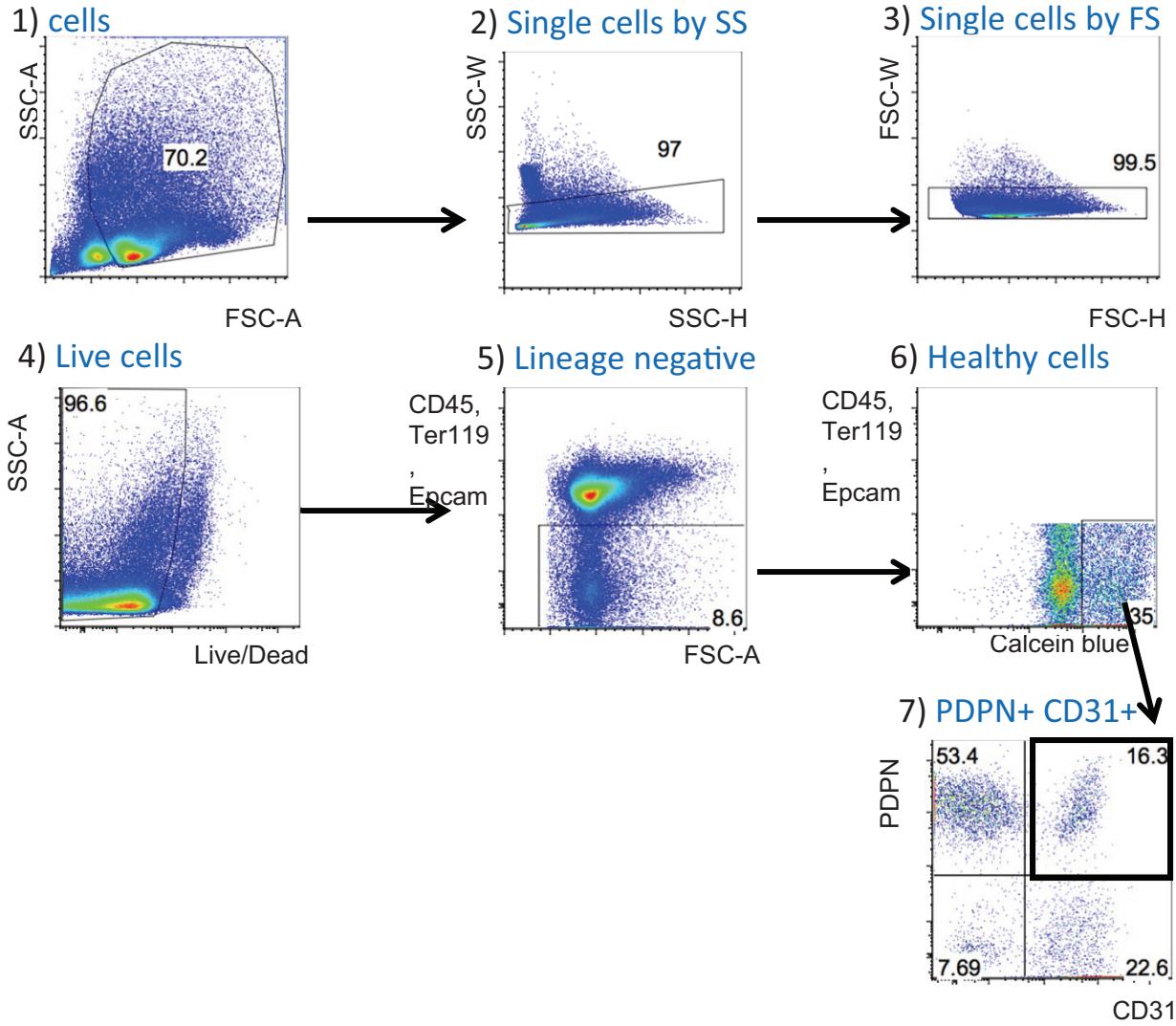
Claudia Dominguez
Turley Lab
Genentech

LEC.SLN: Lymphatic Endothelial cells

Pre-sort



Tissue Preparation:

Dispase and Collagenase P digestion with DNaseI, followed by a depletion employing CD45 microbeads (MACS)

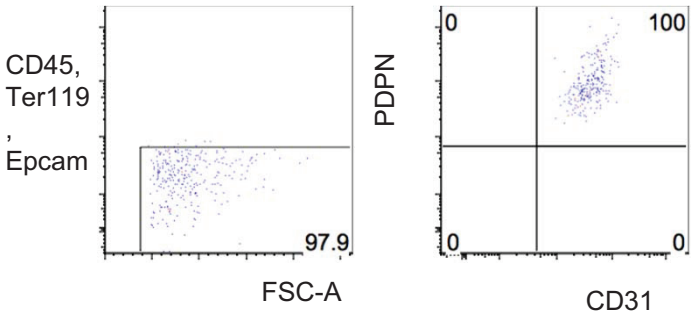
Time from Mouse to the 2nd sorting:

9am to 4pm

Antibody (Clone) - Fluorochrome:

PDPN (8.1.1) -- APC
CD31 (390) -- PE/DAZZLE 594
CD45 (30-F11), Ter119 (TER119), Epcam (G8.8) -- PE-Cy7
Calcein Blue
Live/Dead -- Near-IR633

Post-sort



Purity is 90.1% of cell gate

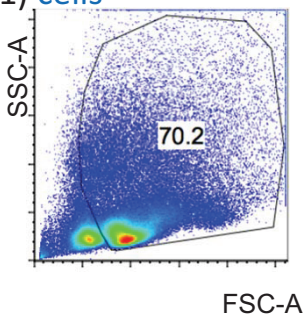
Immunological Genome Project
ImmGen.org

Claudia Dominguez
Turley Lab
Genentech

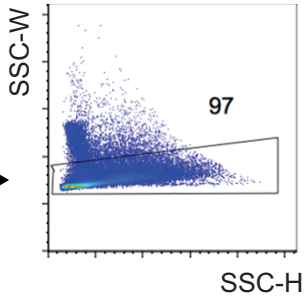
BEC.SLN: Blood Endothelial cells

Pre-sort

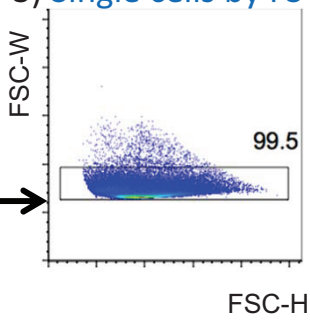
1) cells



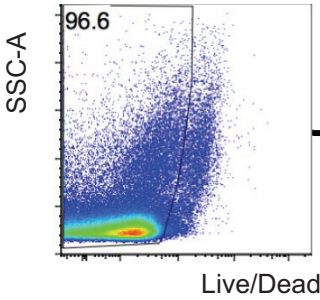
2) Single cells by SS



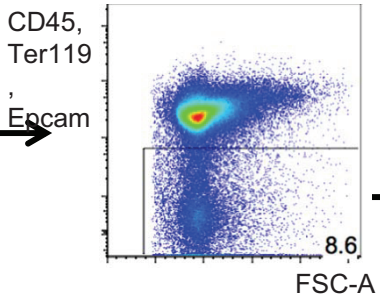
3) Single cells by FS



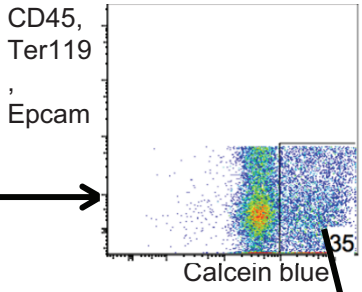
4) Live cells



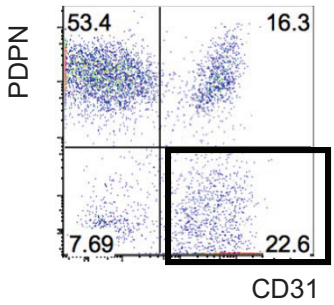
5) Lineage negative



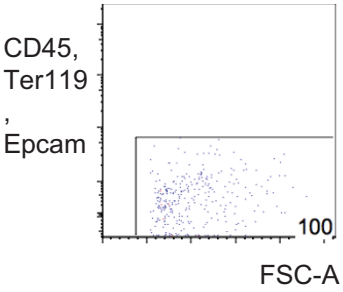
6) Healthy cells

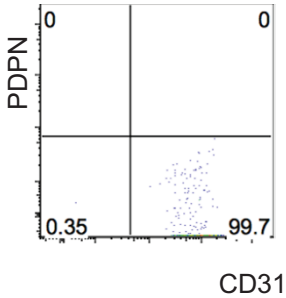


7) PDPN- CD31+



Post-sort





Tissue Preparation:

Dispase and Collagenase P digestion with DNaseI, followed by a depletion employing CD45 microbeads (MACS)

Time from Mouse to the 2nd sorting:

9am to 4pm

Antibody (Clone) - Fluorochrome:

PDPN (8.1.1) -- APC
CD31 (390) -- PE/DAZZLE 594
CD45 (30-F11), Ter119 (TER119), Epcam (G8.8) -- PE-Cy7
Calcein Blue
Live/Dead -- Near-IR633

Purity is 91.4% of cell gate

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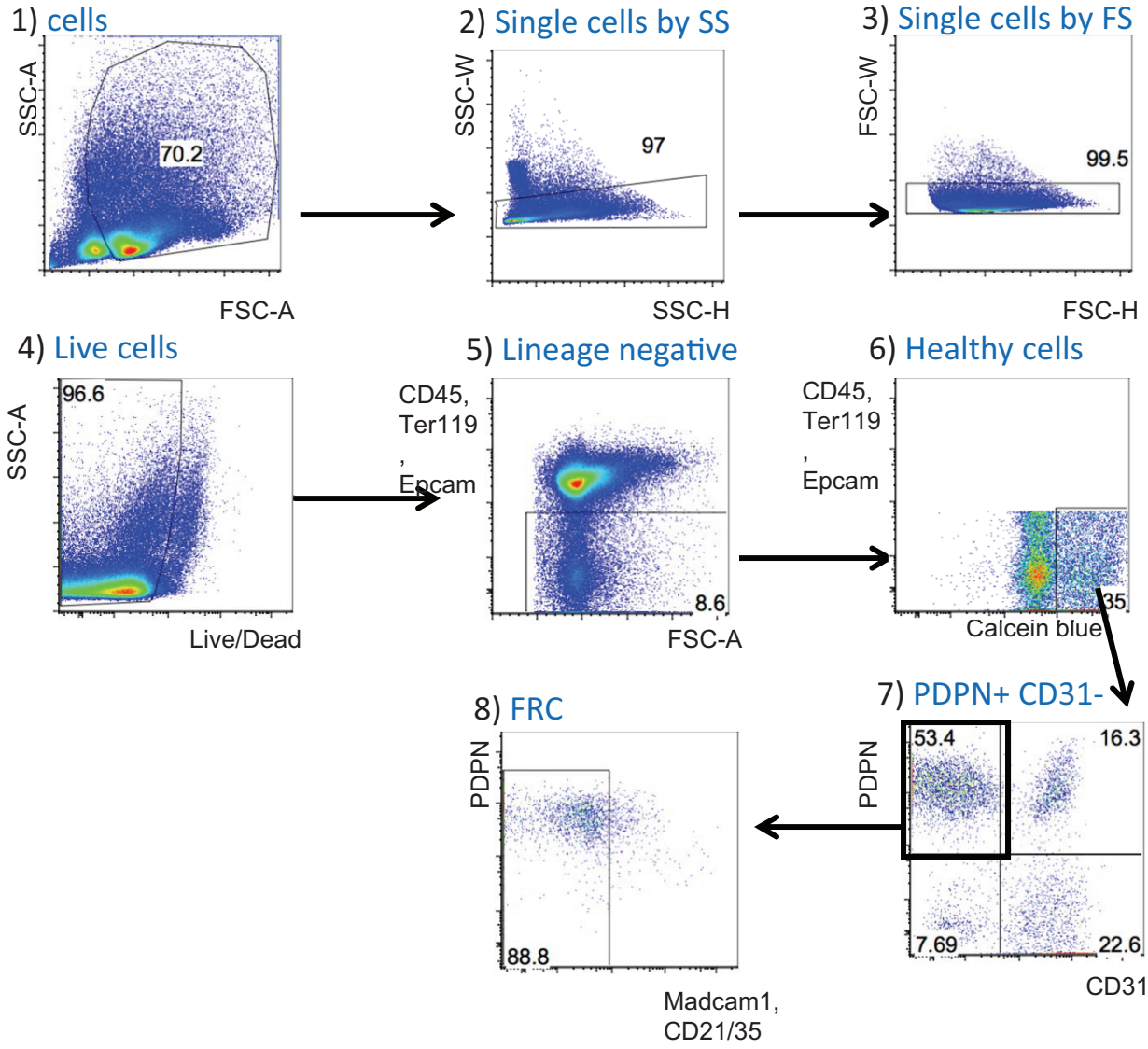
Claudia Dominguez
Turley Lab
Genentech

FRC.SLN: Fibroblastic reticular cells

Pre-sort



Tissue Preparation:

Dispase and Collagenase P digestion with DNaseI, followed by a depletion employing CD45 microbeads (MACS)

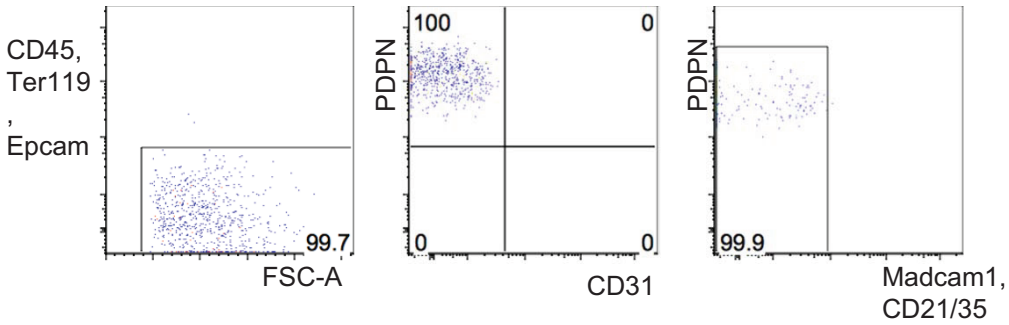
Time from Mouse to the 2nd sorting:

9am to 4pm

Antibody (Clone) - Fluorochrome:

madcam1 (MECA-367) -- Alexa488
CD21/35 (7E9) -- FITC
PDPN (8.1.1) -- APC
CD31 (390) -- PE/DAZZLE 594
CD45 (30-F11), Ter119 (TER119), Epcam (G8.8) -- PE-Cy7
Calcein Blue
Live/Dead -- Near-IR633

Post-sort



Purity is 98.5% of cell gate

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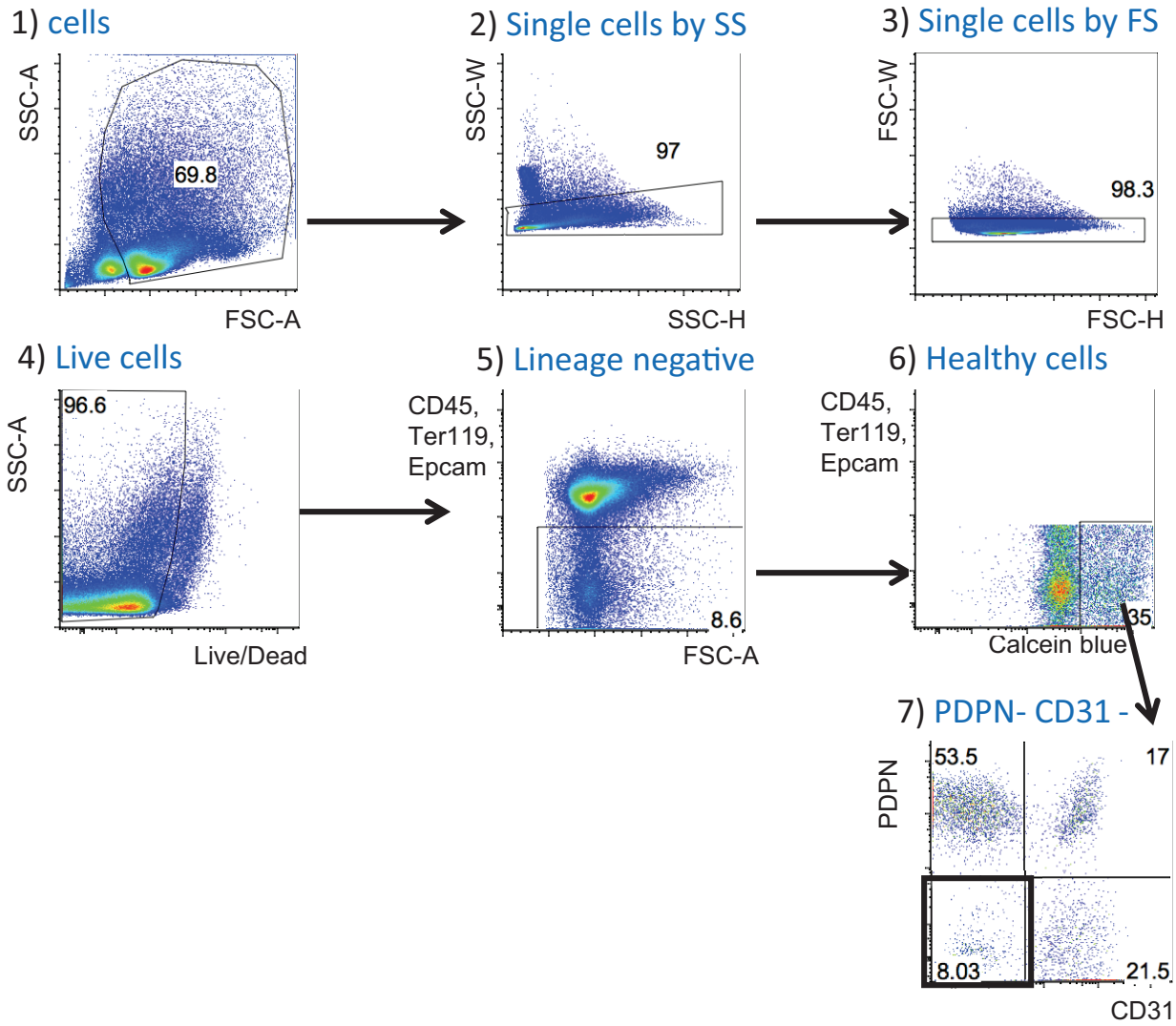
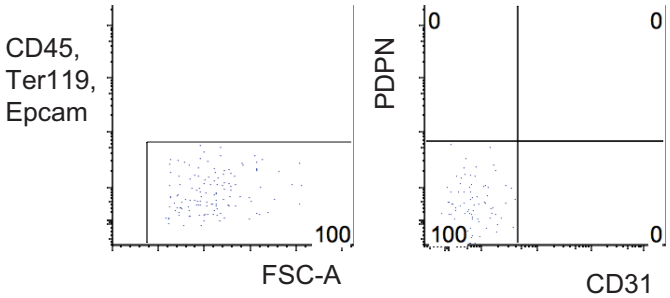
IAP.SLN: Pericytes

Pre-sort



Post-sort



Tissue Preparation:

Dispase and Collagenase P digestion with DNaseI, followed by a depletion employing CD45 microbeads (MACS)

Time from Mouse to the 2nd sorting:

9am to 4pm

Antibody (Clone) - Fluorochrome:

PDPN (8.1.1) -- APC
CD31 (390) -- PE/DAZZLE 594
CD45 (30-F11), Ter119 (TER119), Epcam (G8.8) -- PE-Cy7
Calcein Blue
Live/Dead -- Near-IR633

Purity is 99.2% of cell gate