# *Simultaneous correlation of cytokine production with Treg and Th17 cell proliferation*

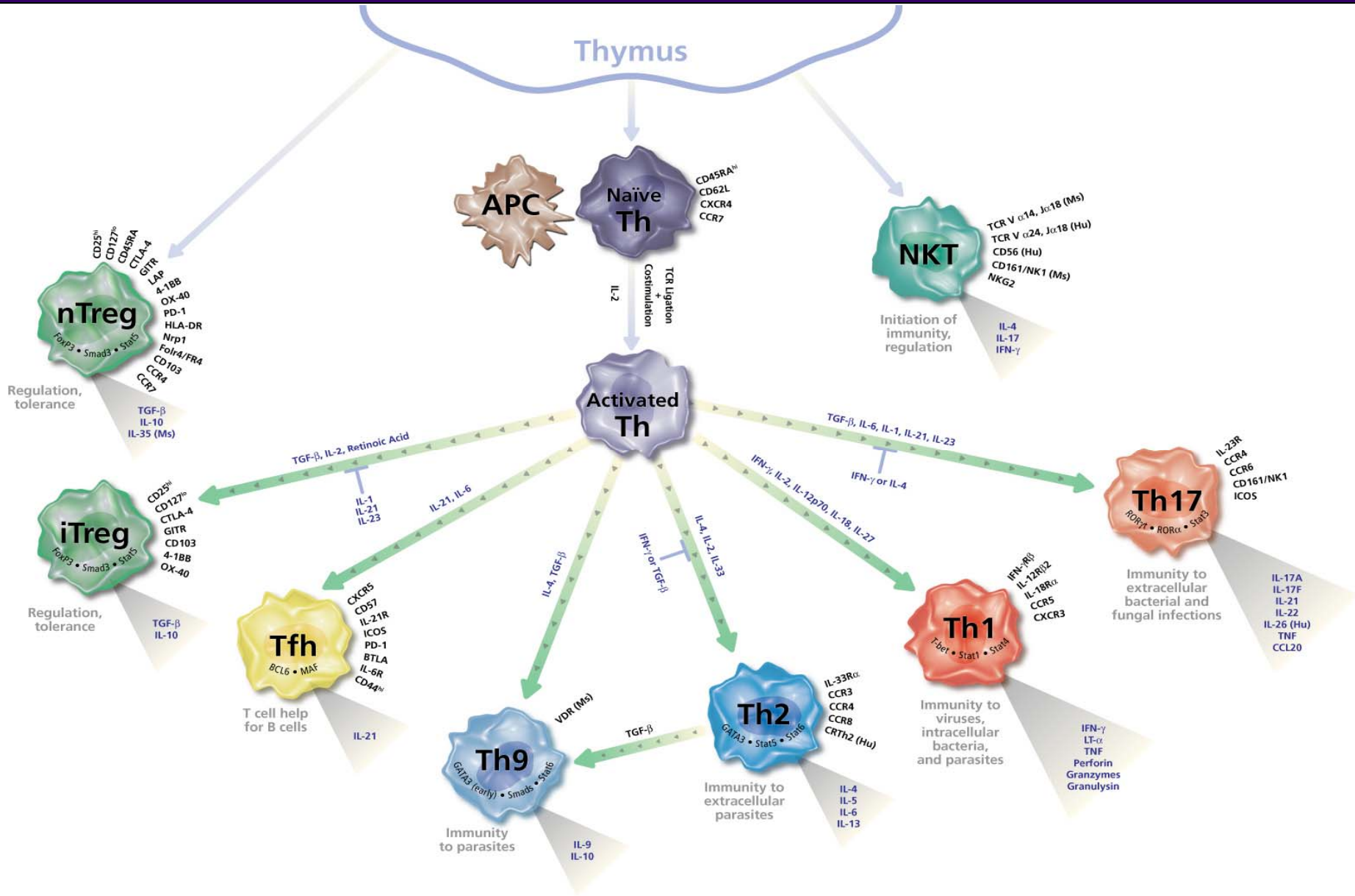
Jurg Rohrer, PhD
Director, R&D
BD Biosciences

23-11773-00

# Overview

- T helper (Th) cell overview
- Experimental setup
- Data analysis
- Conclusions

# Introduction to Th biology

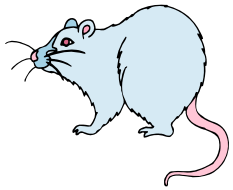


# *Experimental setup*

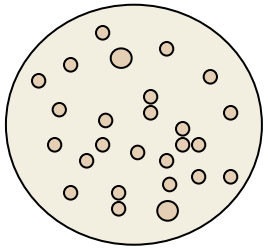
- Enrich Balb/c splenocytes by positive selection via $CD4^+$ panning
- Load isolated cells with VFSE 1μM, 10 minutes
- Set up cultures as follows:
  - CD3/CD28
  - CD3/CD28/IL-6/IL-1β
  - CD3/CD28/IL-6/IL-1β/TGF-β
  - CD3/CD28/IL-6/IL-1β/TGF-β/IL-23
- Harvest cells at 1, 2, 3, and 4 days
- Fix/perm and stain cells for IL-17A, Foxp3, IL-4, IL-2, and interferon-γ (IFN-γ)

BD

# Experimental setup continued



Harvest
Spleen



CD4 cells enriched
by panning

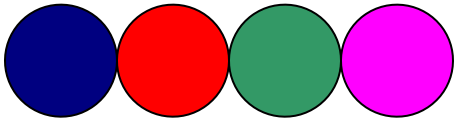
Cells loaded with
VFSE and washed

anti-CD3e+
anti-CD28

anti-CD3e+
anti-CD28+IL-1β+IL-6

anti-CD3e+
anti-CD28+IL-1β+IL-6+TGF-β

anti-CD3e+
anti-CD28+IL-1β+IL-6+TGF-β+ IL-23



Day 1, 2, 3, 4

BD Cytofix/Cytoperm™ buffer
Cytokines

+ Monensin

Foxp3 Fix/Perm buffer
Foxp3 and some
cytokines

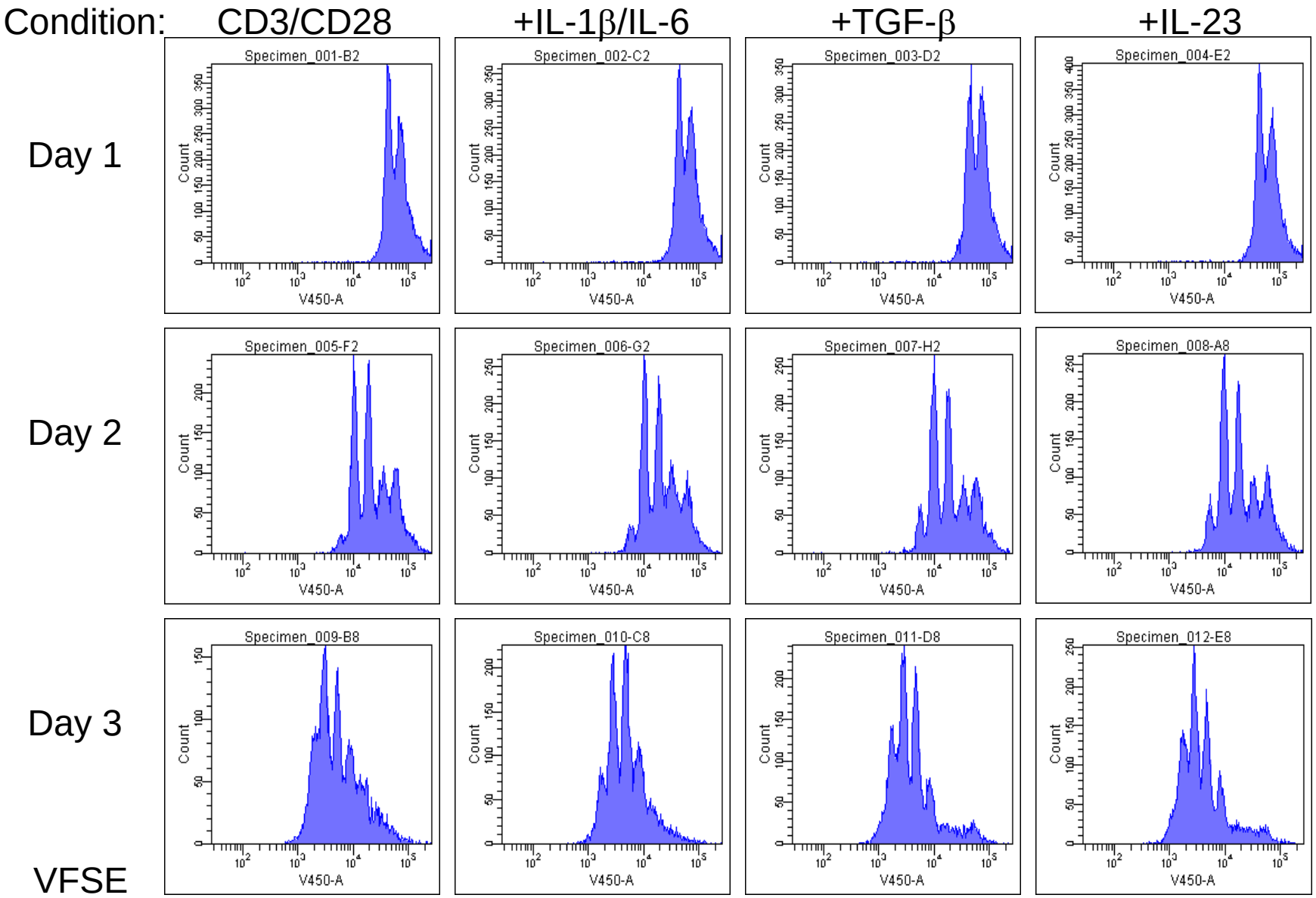
Harvest
Stimulate with PMA and
Ionomycin for 4–5 hours
+ or – Monensin

- Monensin

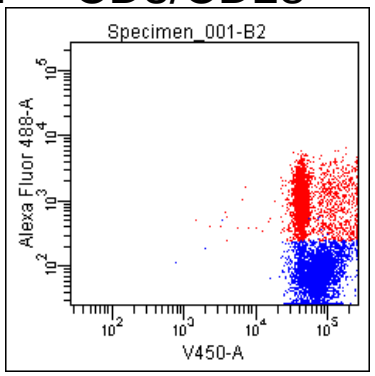
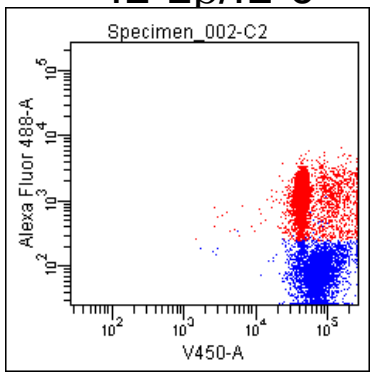
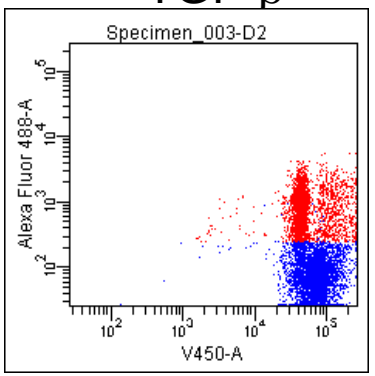
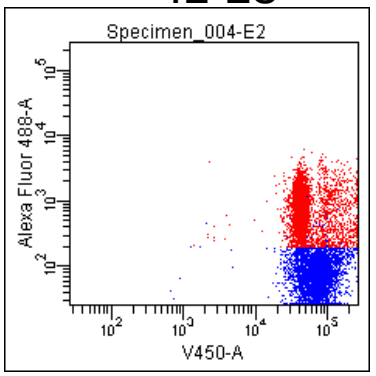
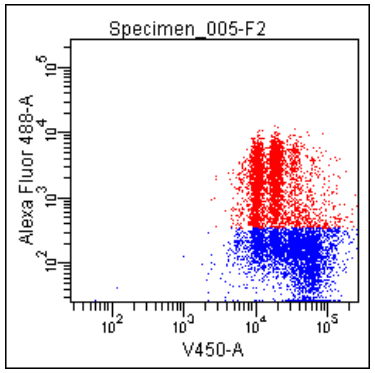
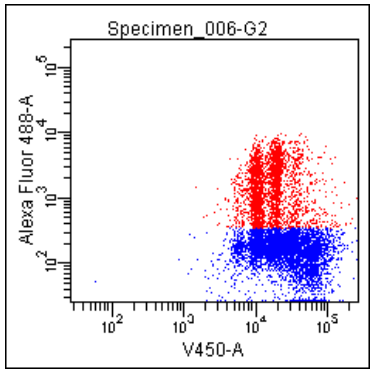
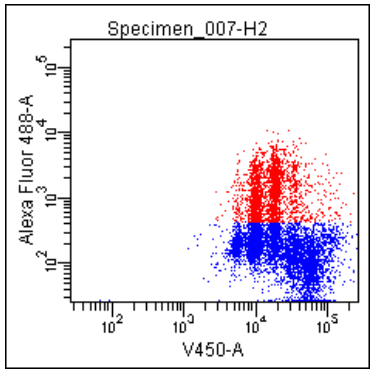
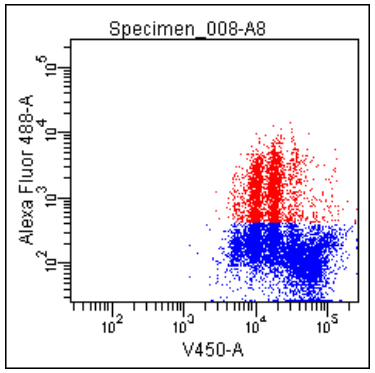
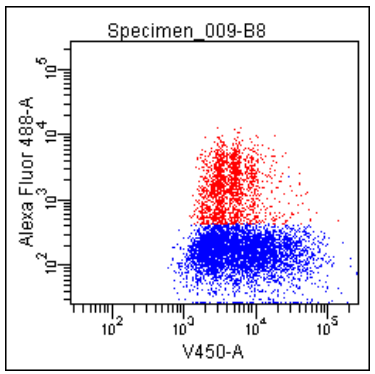
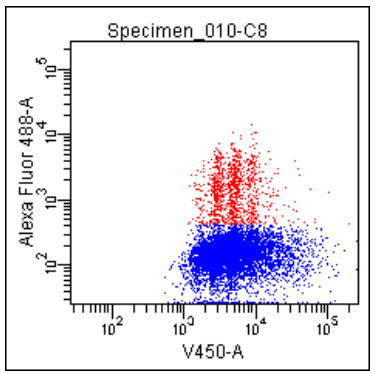
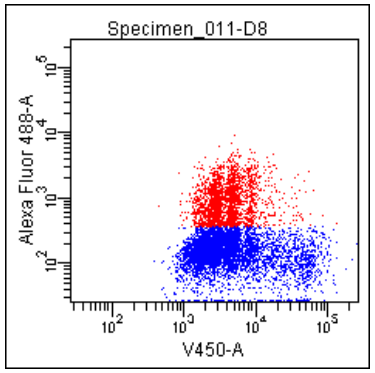
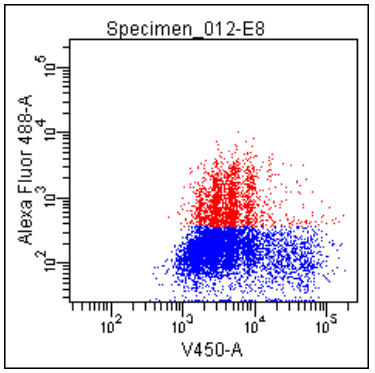
Supernatants: Analysis with BD™ CBA Flex Sets
Cells: BD™ Phosflow Fix and Perm buffer

BD

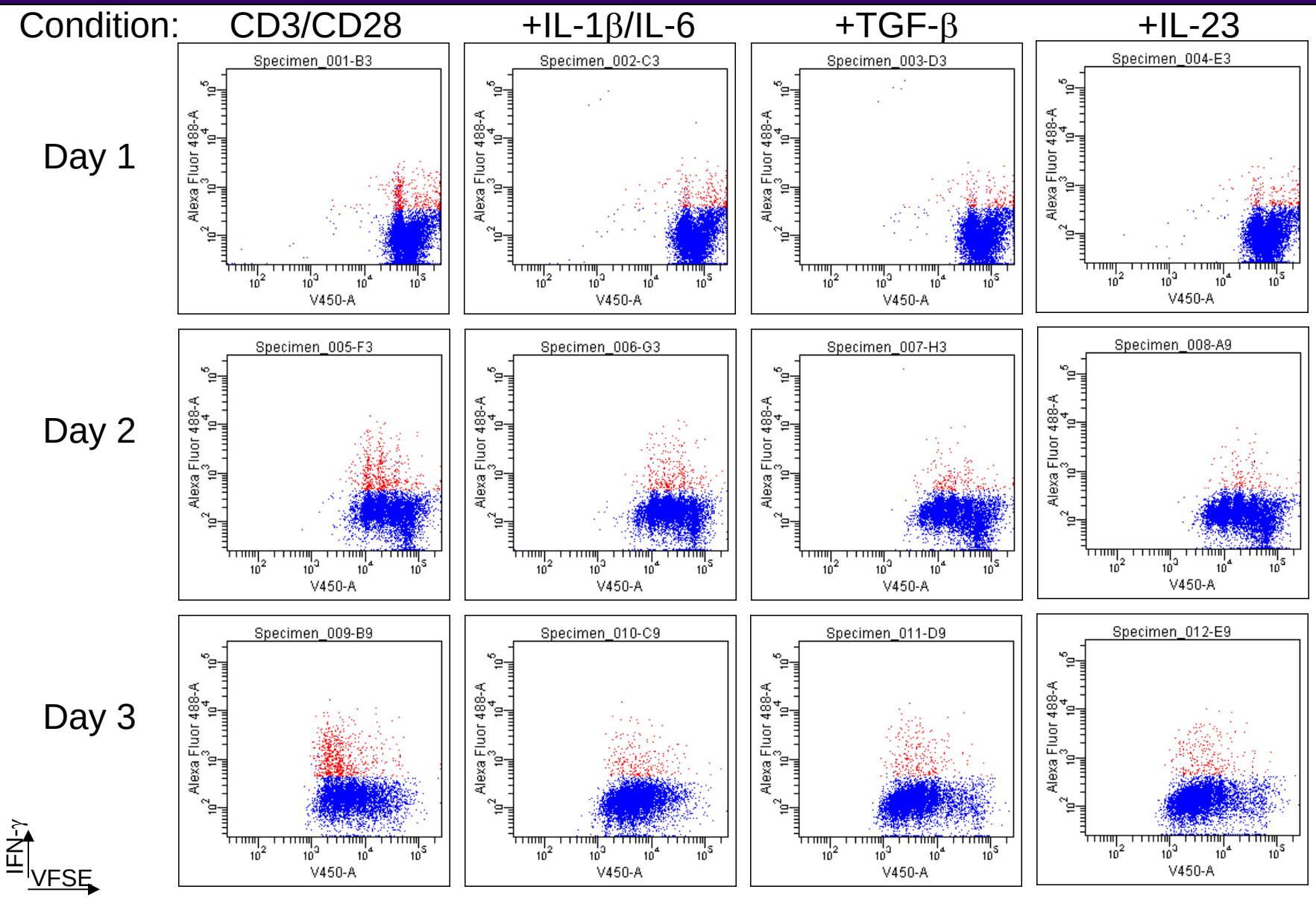
# *VFSE histograms*



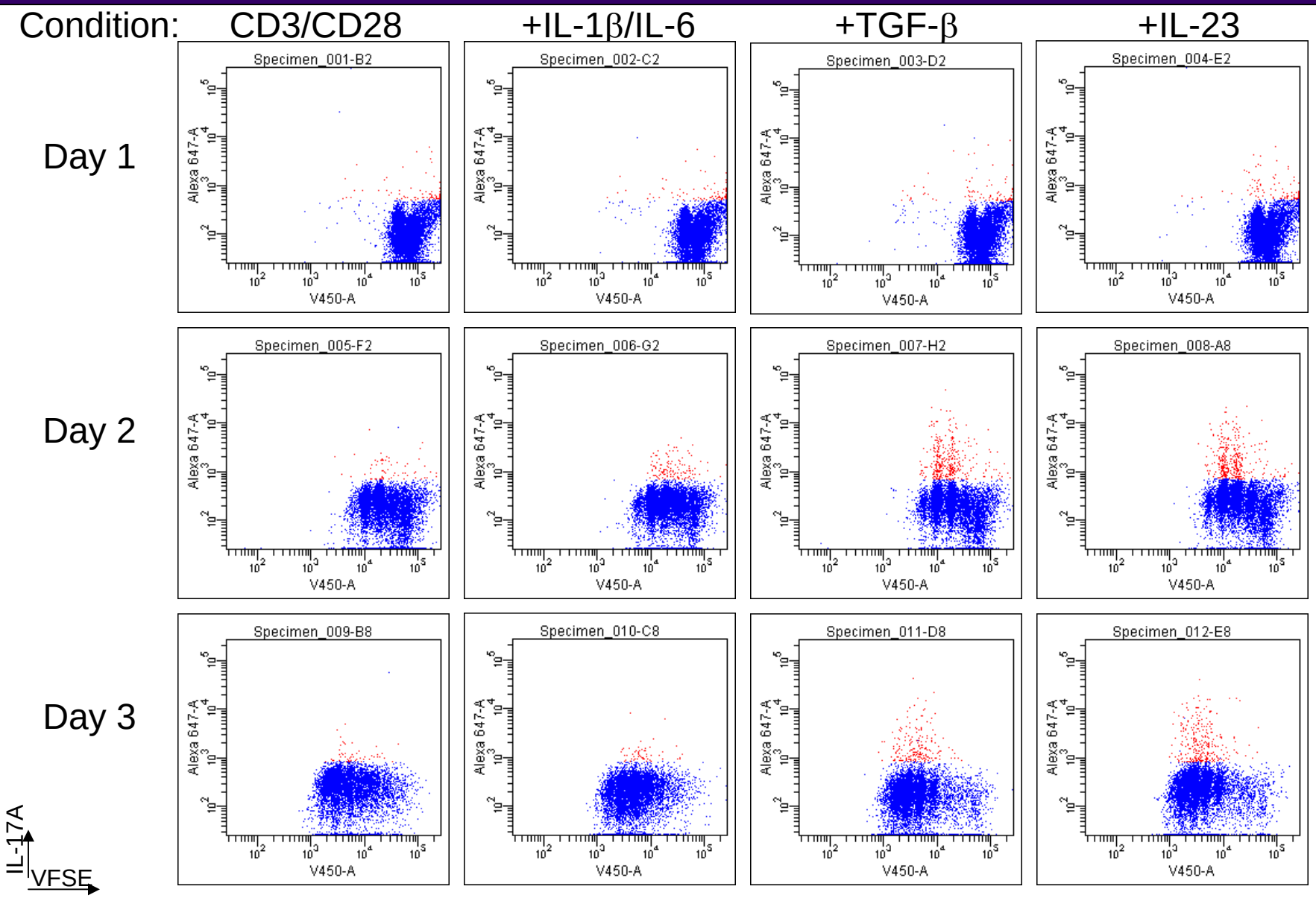
# VFSE vs IL-2 data

| Condition: | CD3/CD28 | +IL-1β/IL-6 | +TGF-β | +IL-23 |
|---|---|---|---|---|
| Day 1 |  |  |  |  |
| Day 2 |  |  |  |  |
| Day 3 |  |  |  |  |

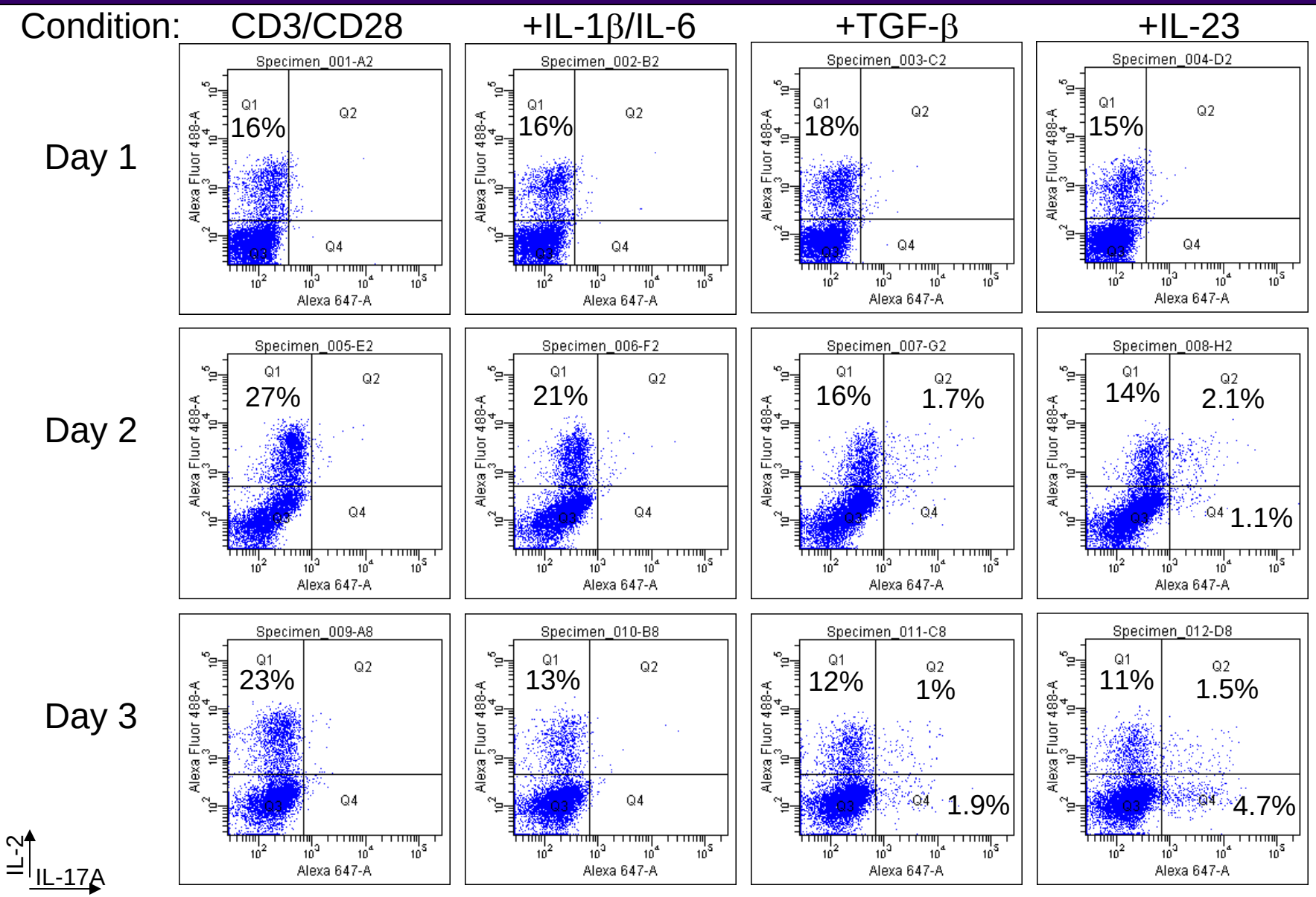
# VFSE vs IFN-γ data



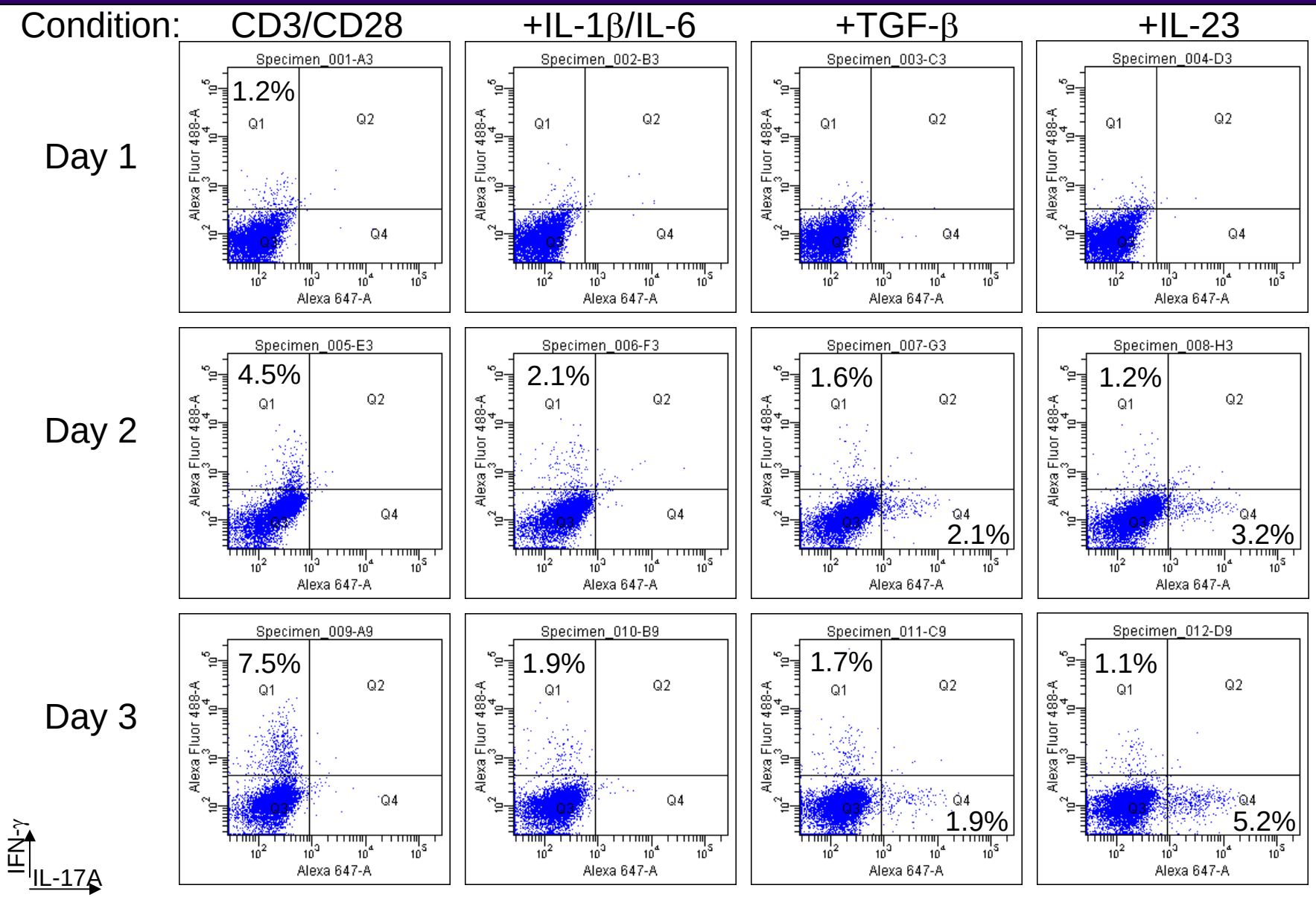
# VFSE vs IL-17A data



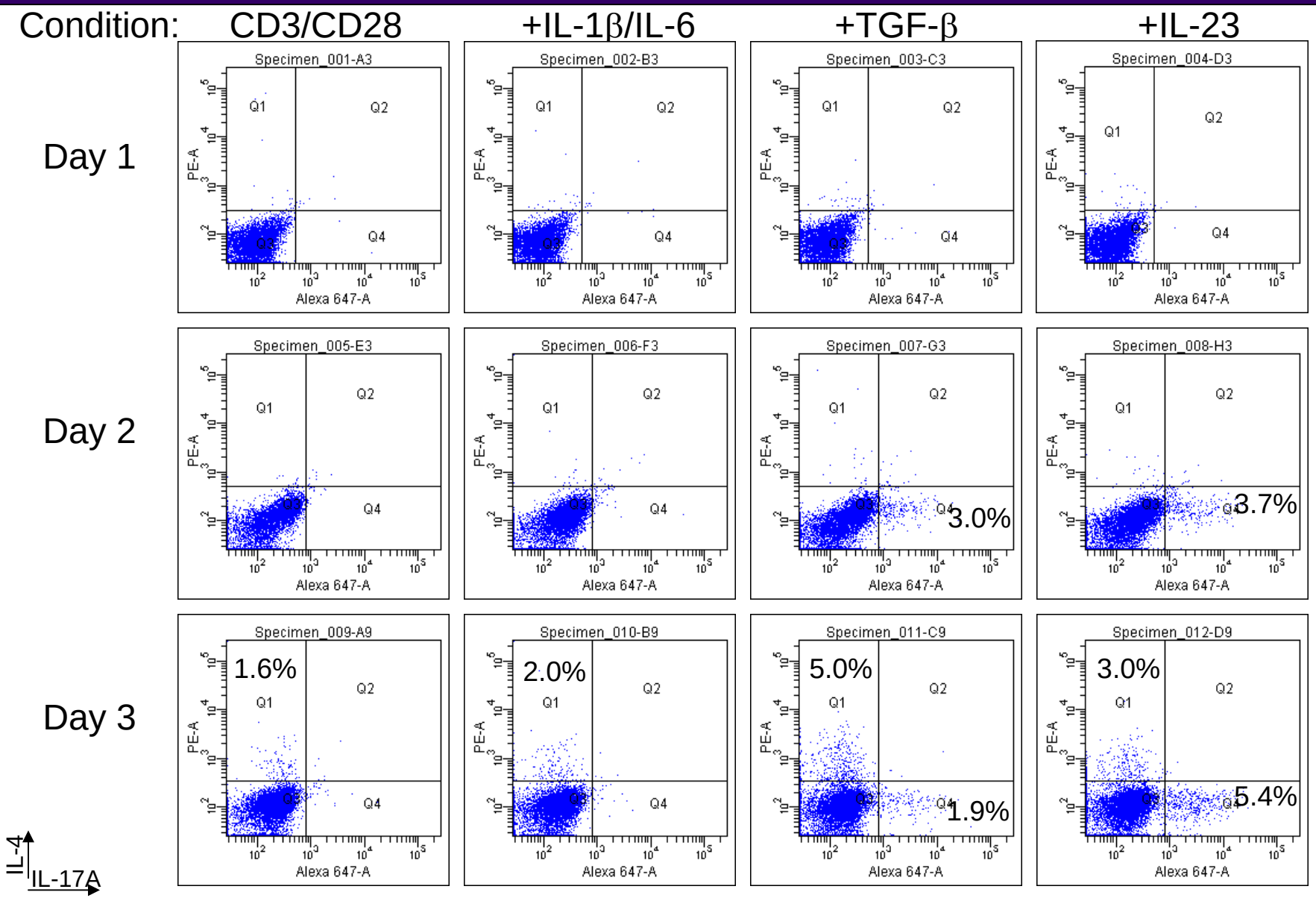
# *Co-expression of IL-17A vs IL-2*



# Co-expression of IL-17A vs IFN-γ

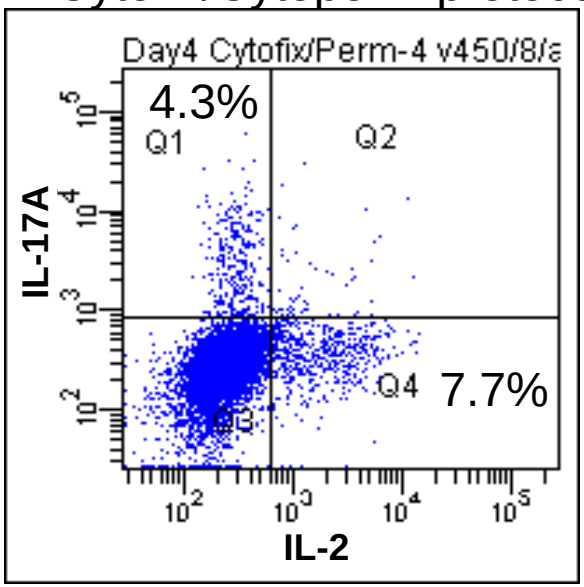


# *Co-expression of IL-17A vs IL-4*

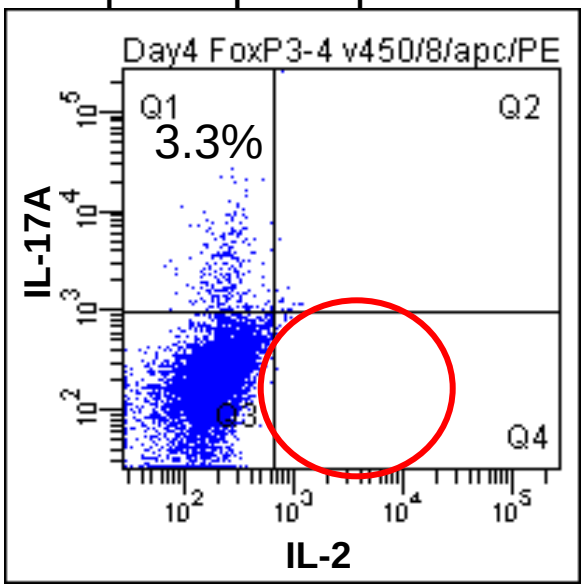


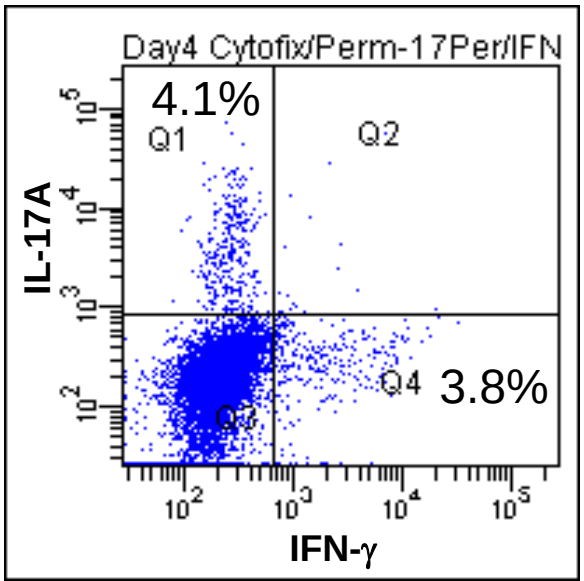
# Comparison of two fix/perm protocols

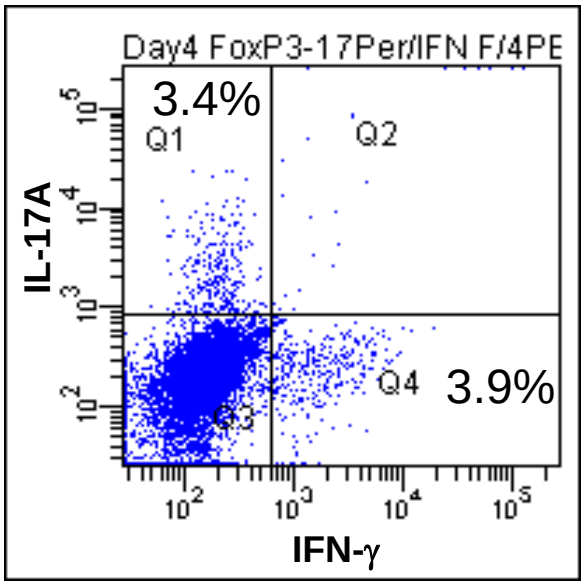
BD Cytofix/Cytoperm protocol



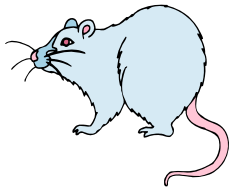

Foxp3 fix/perm protocol



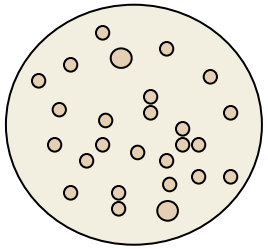



# Experimental setup



Harvest
Spleen



CD4 cells enriched
by panning

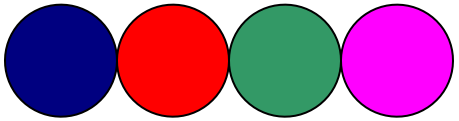
Cells loaded with
VFSE and washed

anti-CD3e+
anti-CD28

anti-CD3e+
anti-CD28+IL-1β+IL-6

anti-CD3e+
anti-CD28+IL-1β+IL-6+TGF-β

anti-CD3e+
anti-CD28+IL-1β+IL-6+TGF-β+ IL-23



Day 1, 2, 3, 4

BD Cytofix/Cytoperm™ buffer
Cytokines

+ Monensin

Foxp3 Fix/Perm buffer
Foxp3 and some
cytokines

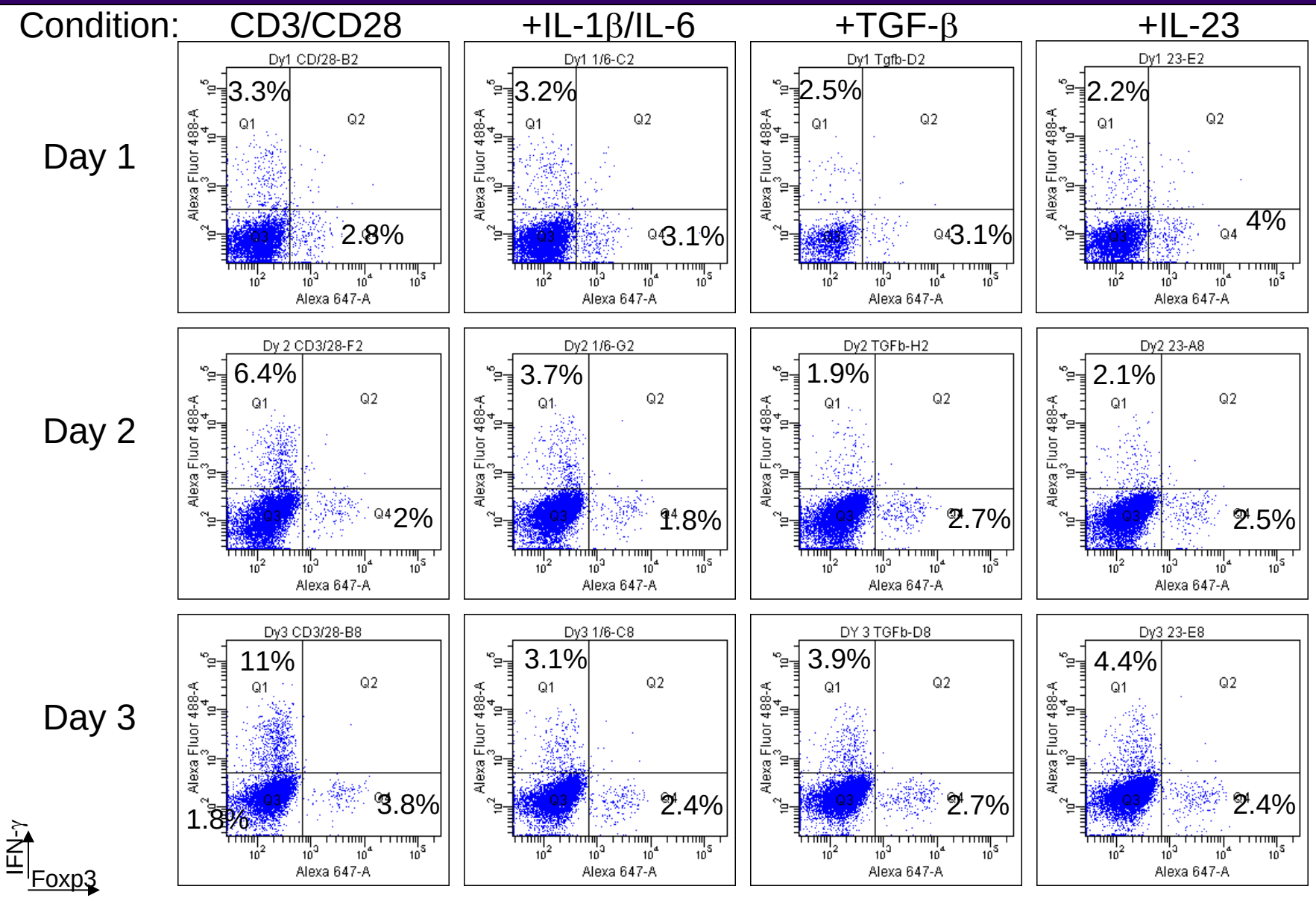
Harvest
Stimulate with PMA and
Ionomycin for 4–5 hours
+ or – Monensin

- Monensin

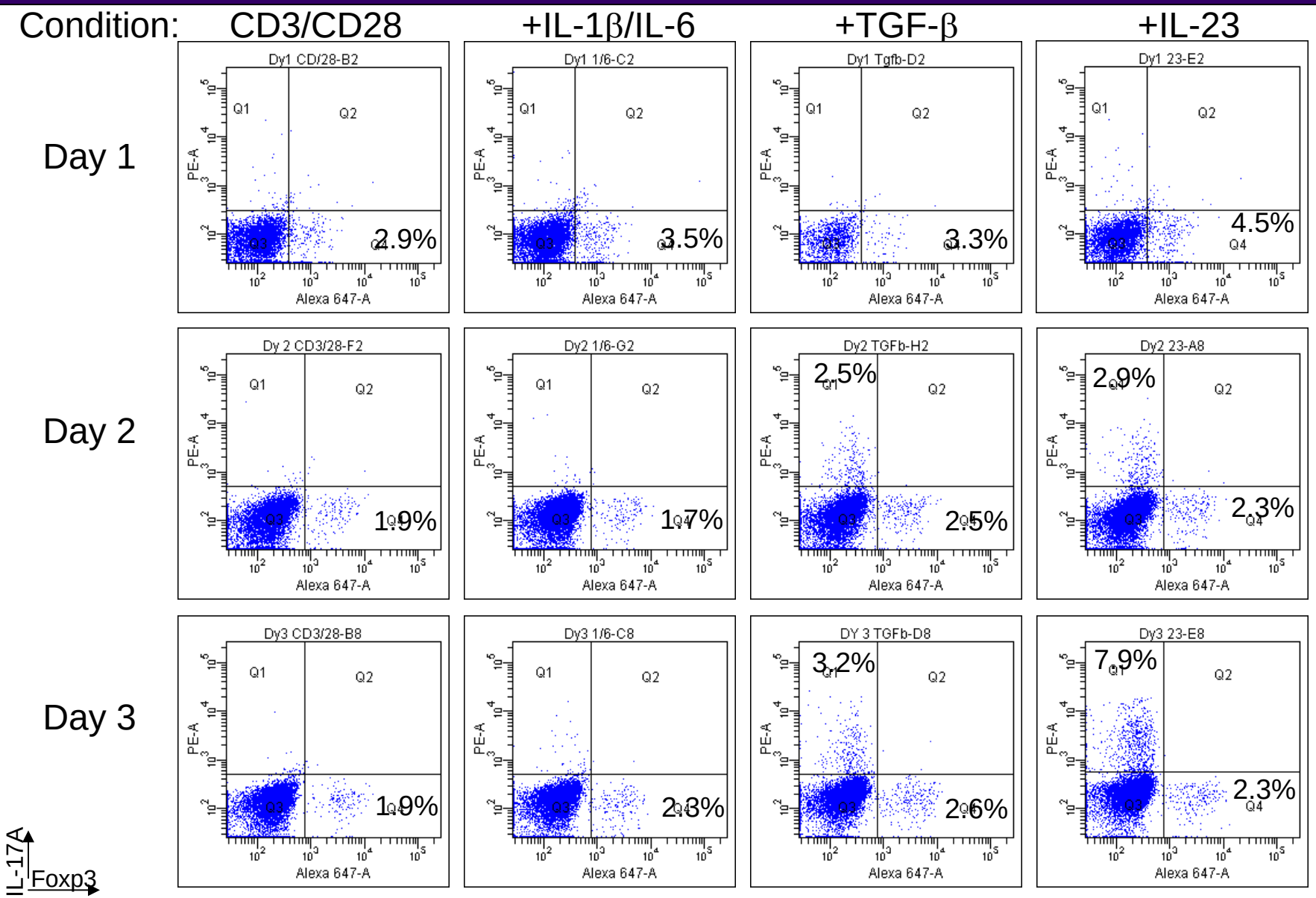
Supernatants: Analysis with BD™ CBA Flex Sets
Cells: BD™ Phosflow Fix and Perm buffer

BD

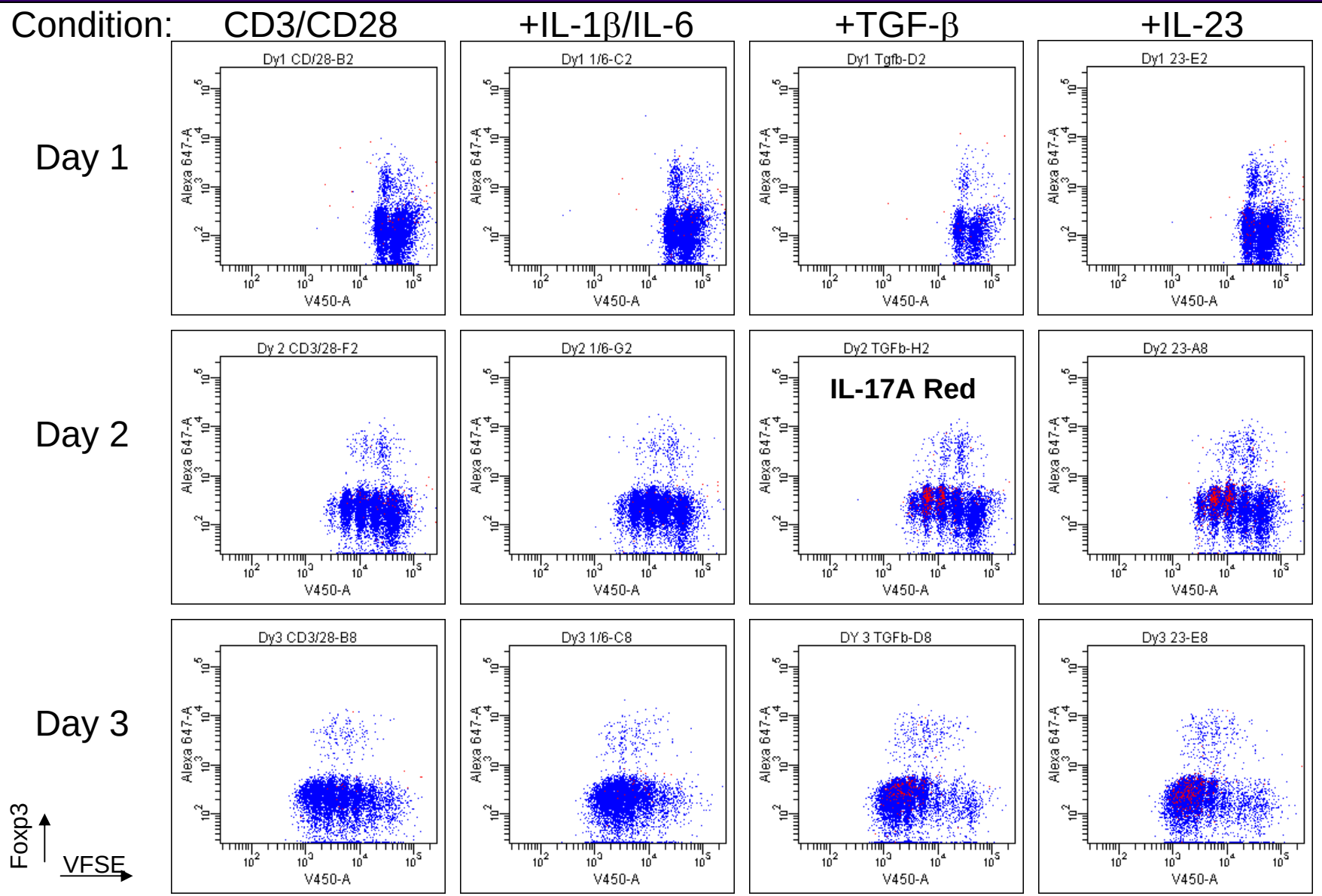
# Co-expression of Foxp3 vs IFN-γ



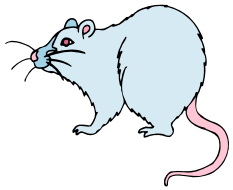
# *Co-expression of Foxp3 vs IL-17A*



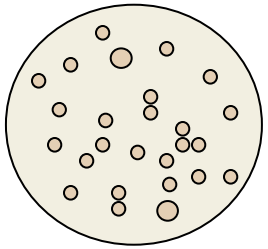
# *Proliferation of Treg and Th17 cells*



# Experimental setup



Harvest spleen



CD4 cells enriched by panning

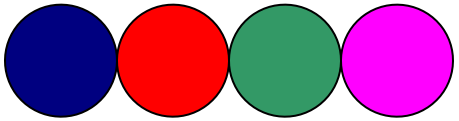
Cells loaded with VFSE and washed

anti-CD3e+ anti-CD28

anti-CD3e+ anti-CD28+IL-1β+IL-6

anti-CD3e+ anti-CD28+IL-1β+IL-6+TGF-β

anti-CD3e+ anti-CD28+IL-1β+IL-6+TGF-β+IL-23



Day 1, 2, 3, 4

+ Monensin

BD Cytofix/Cytoperm buffer
Cytokines

Foxp3 Fix/Perm buffer
Foxp3 and some cytokines

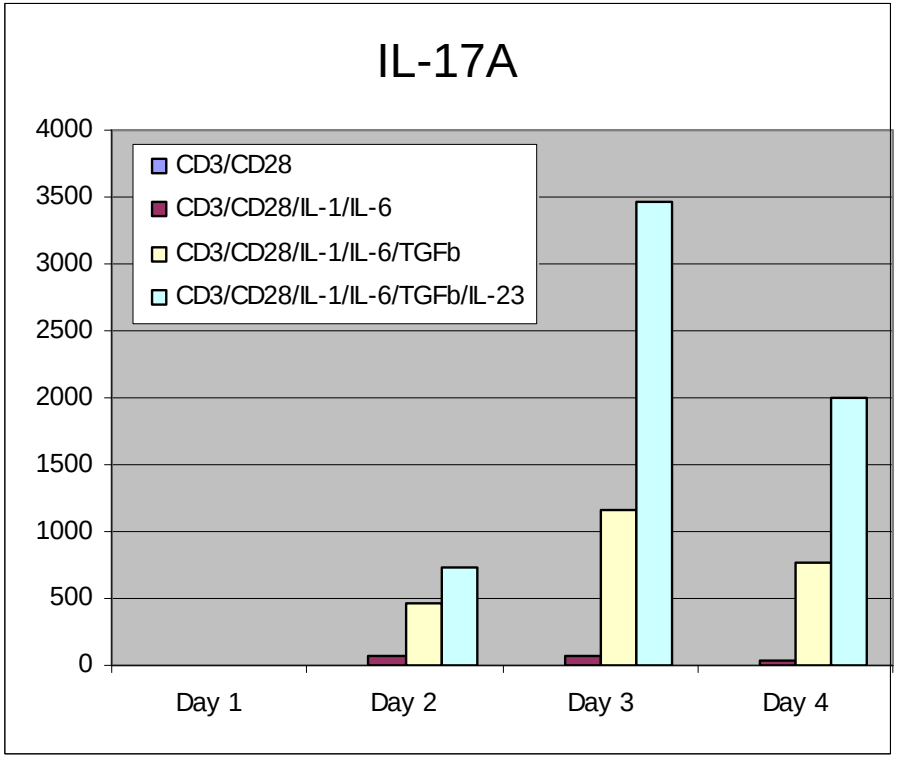
Harvest
Stimulate with PMA and Ionomycin for 4–5 hours
+ or – Monensin

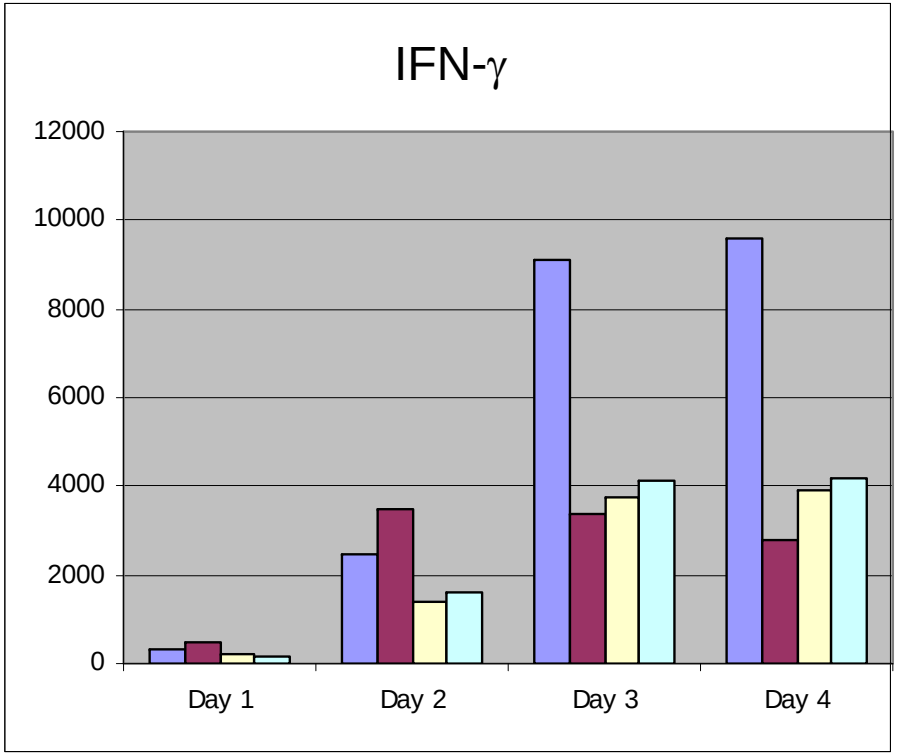
- Monensin

Supernatants: Analysis with BD CBA Flex Sets
Cells: BD Phosflow Fix and Perm buffer

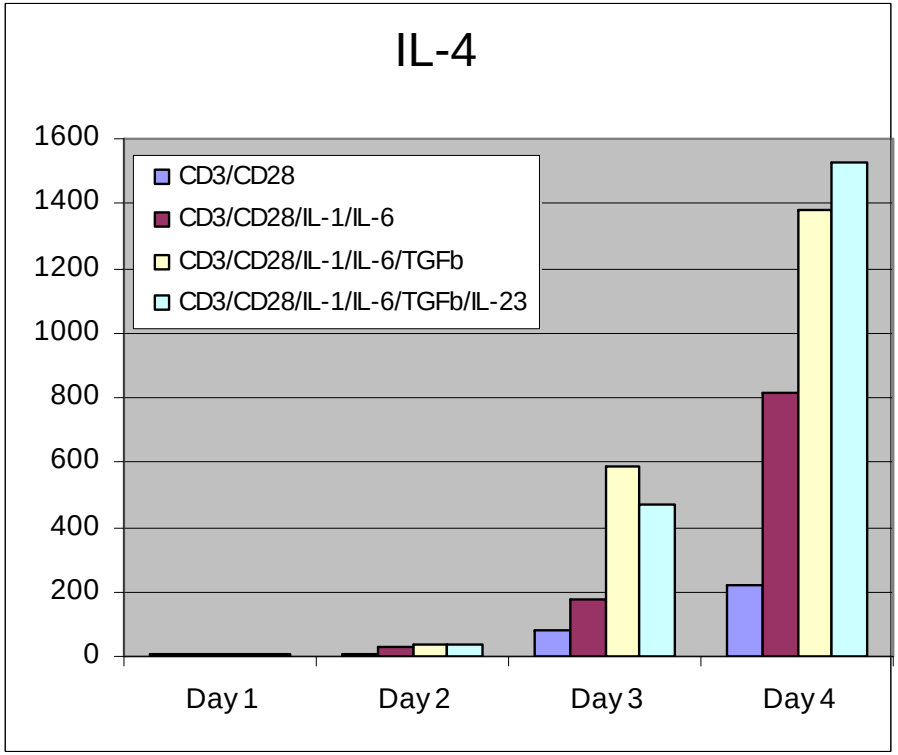
BD

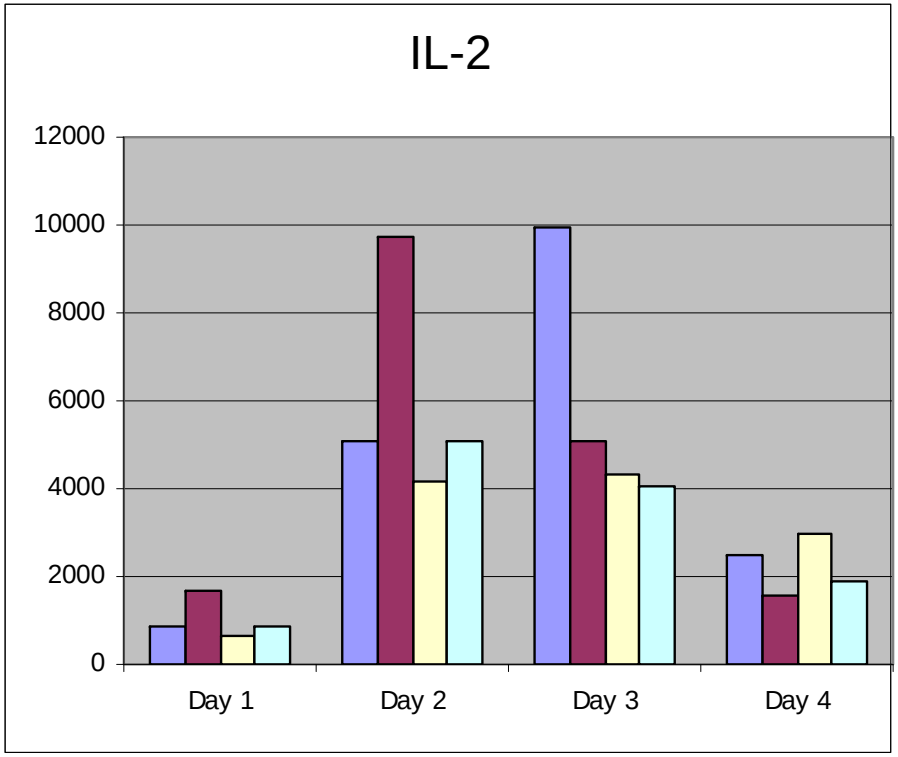
# IL-17A and IFN-γ production



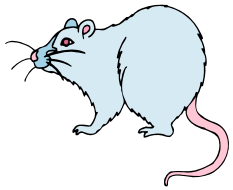
# IL-4 and IL-2 production



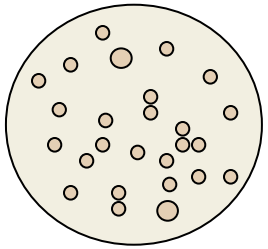




BD

# Experimental setup



Harvest spleen



CD4 cells enriched by panning

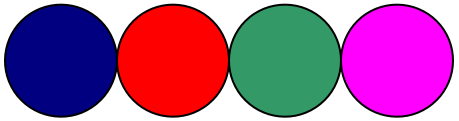
Cells loaded with VFSE and washed

anti-CD3e+ anti-CD28

anti-CD3e+ anti-CD28+IL-1β+IL-6

anti-CD3e+ anti-CD28+IL-1β+IL-6+TGF-β

anti-CD3e+ anti-CD28+IL-1β+IL-6+TGF-β+IL-23



Day 1, 2, 3, 4

+ Monensin

BD Cytofix/Cytoperm buffer
Cytokines

Foxp3 Fix/Perm buffer
Foxp3 and some cytokines

Harvest
Stimulate with PMA and Ionomycin for 4–5 hours
+ or – Monensin

- Monensin

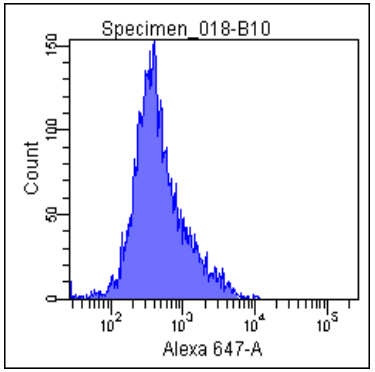
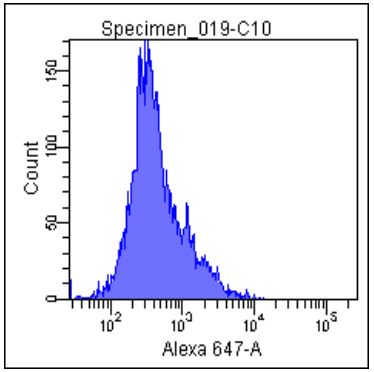
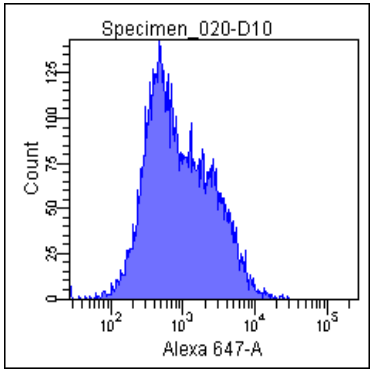
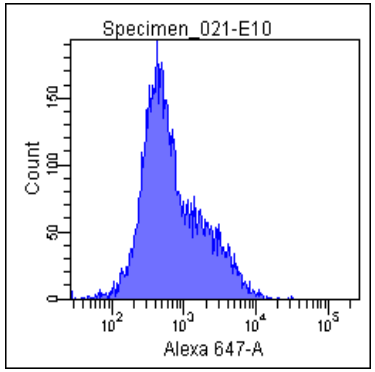
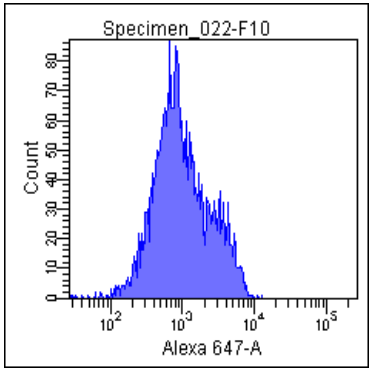
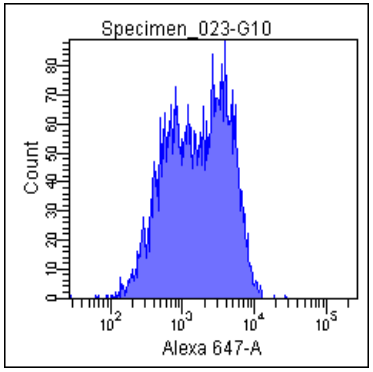
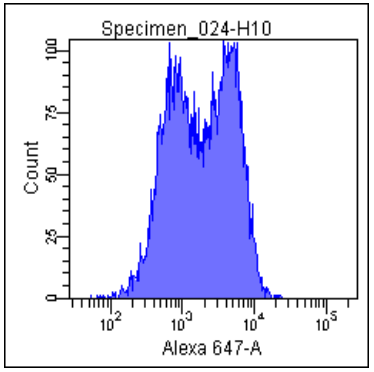
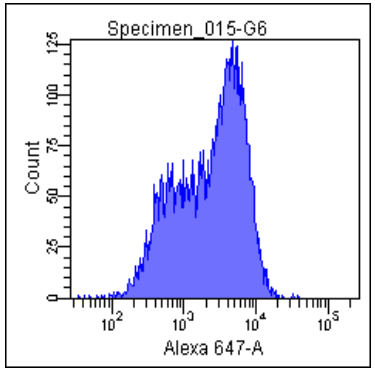
Supernatants: Analysis with BD CBA Flex Sets
Cells: BD Phosflow Fix and Perm buffer

BD

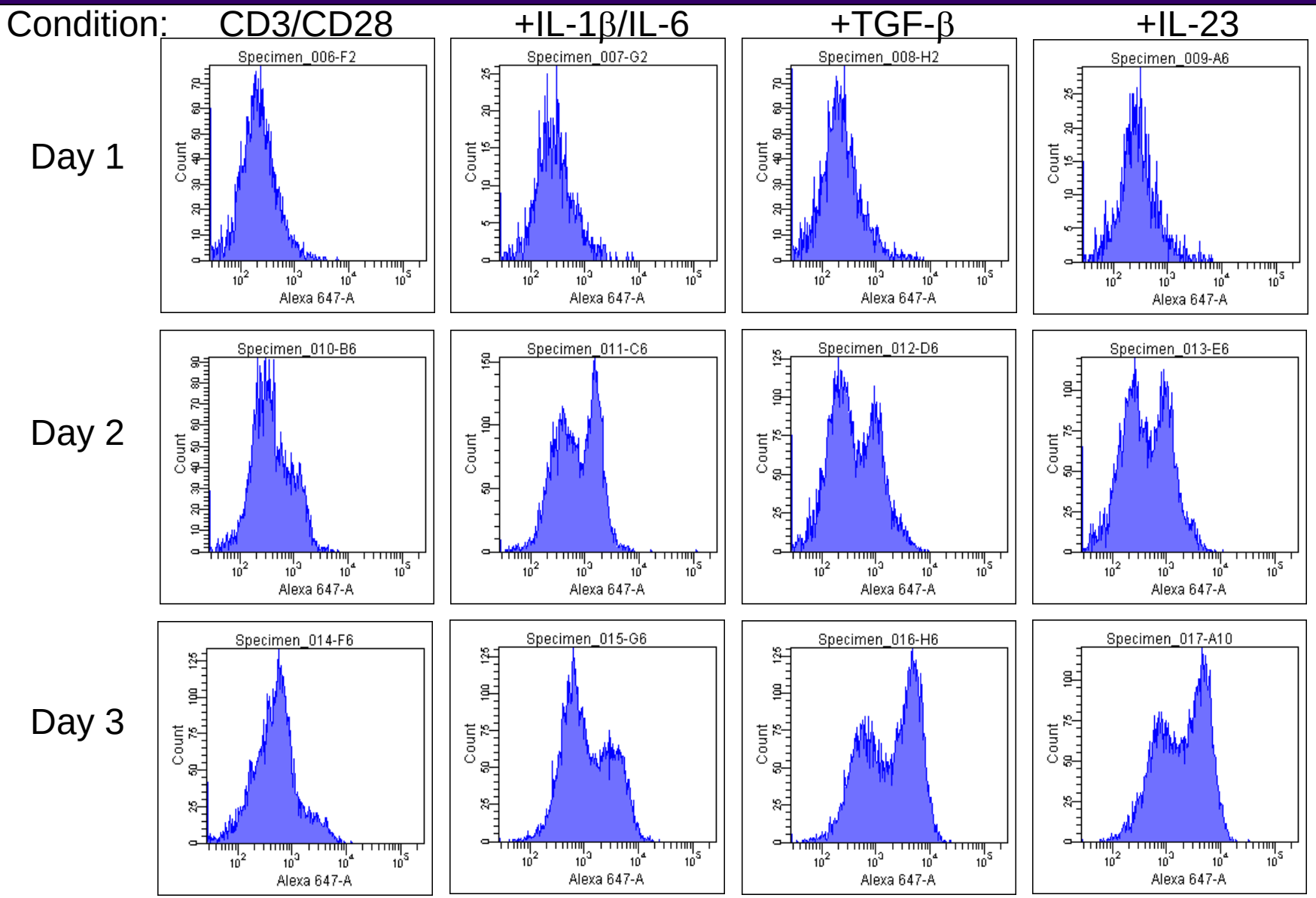
# *pStat5 detection on day 4*

**Unactivated:** Cells were cultured, harvested, and stained with phosphospecific Stat5 antibody.

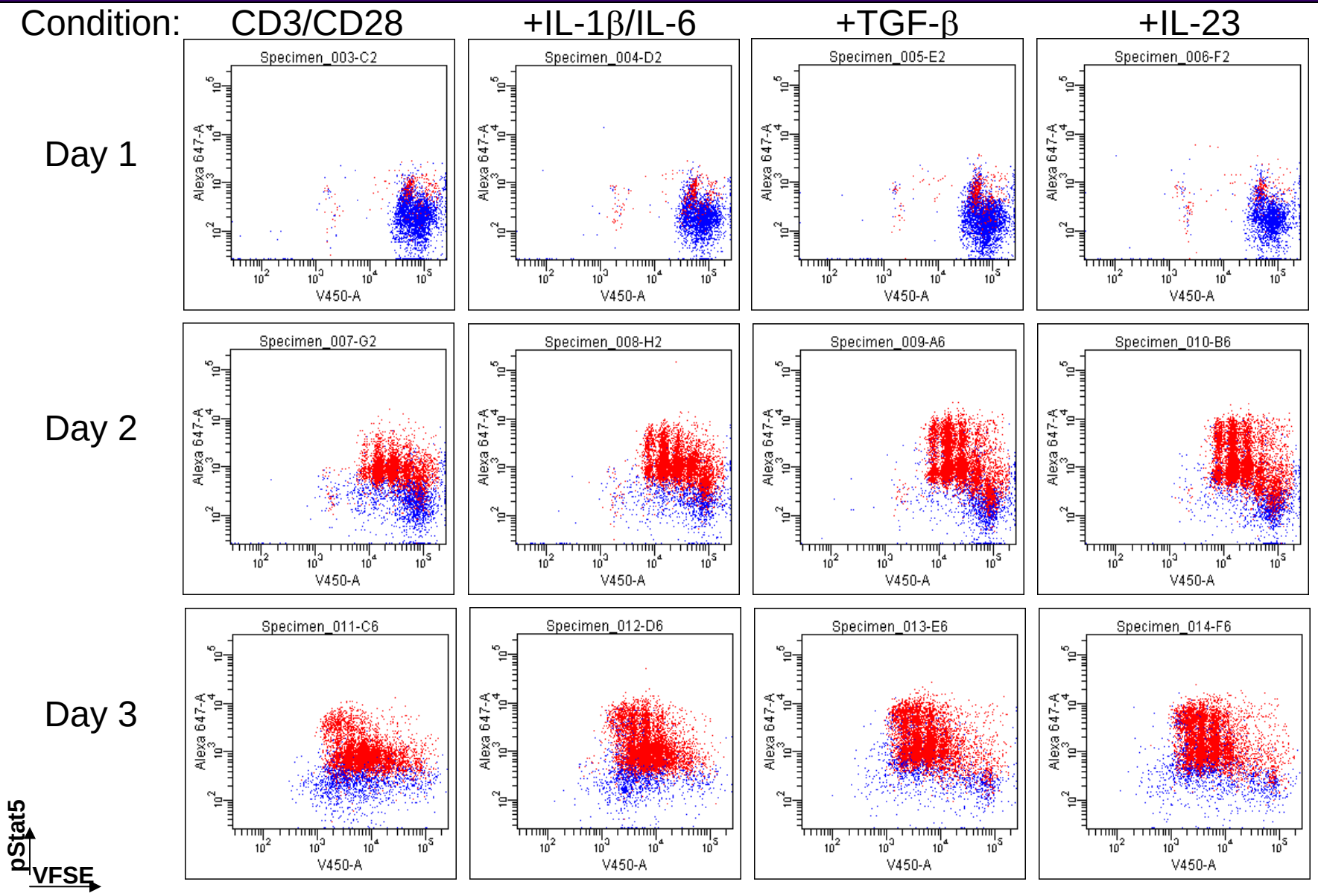
**Activated:** Cells were cultured and activated with PMA/Ionomycin for 5 hours and then stained with phosphospecific Stat5 antibody.

| Condition: | CD3/CD28 | +IL-1β/IL-6 | +TGF-β | +IL-23 |
|---|---|---|---|---|
| Unactivated |   |   |   |   |
| Activated |   |   |   |   |

# *pStat5 in activated cells over time*



# *pStat5 in proliferating cells*



# Conclusions

- Cells proliferated equally well under all four polarization conditions
- Invitro cultures showed that TGF-β was important for polarization of CD4 cells towards Th17
- Initial cultures show co-expression of IL-2 and IL-17 that later become independent of each other
- Detection of secreted cytokines (by CBA) correlated with the intracellular staining
- Cytokine production by proliferating cells resulted in increased phosphorylation of the signal transducer Stat5

BD

# Acknowledgments


- Jeanne Elia
- Xiao-Wei Wu
- Ravi Hingorani

- Jacob Rabenstein