Simultaneous correlation of cytokine production with Treg and Th17 cell proliferation

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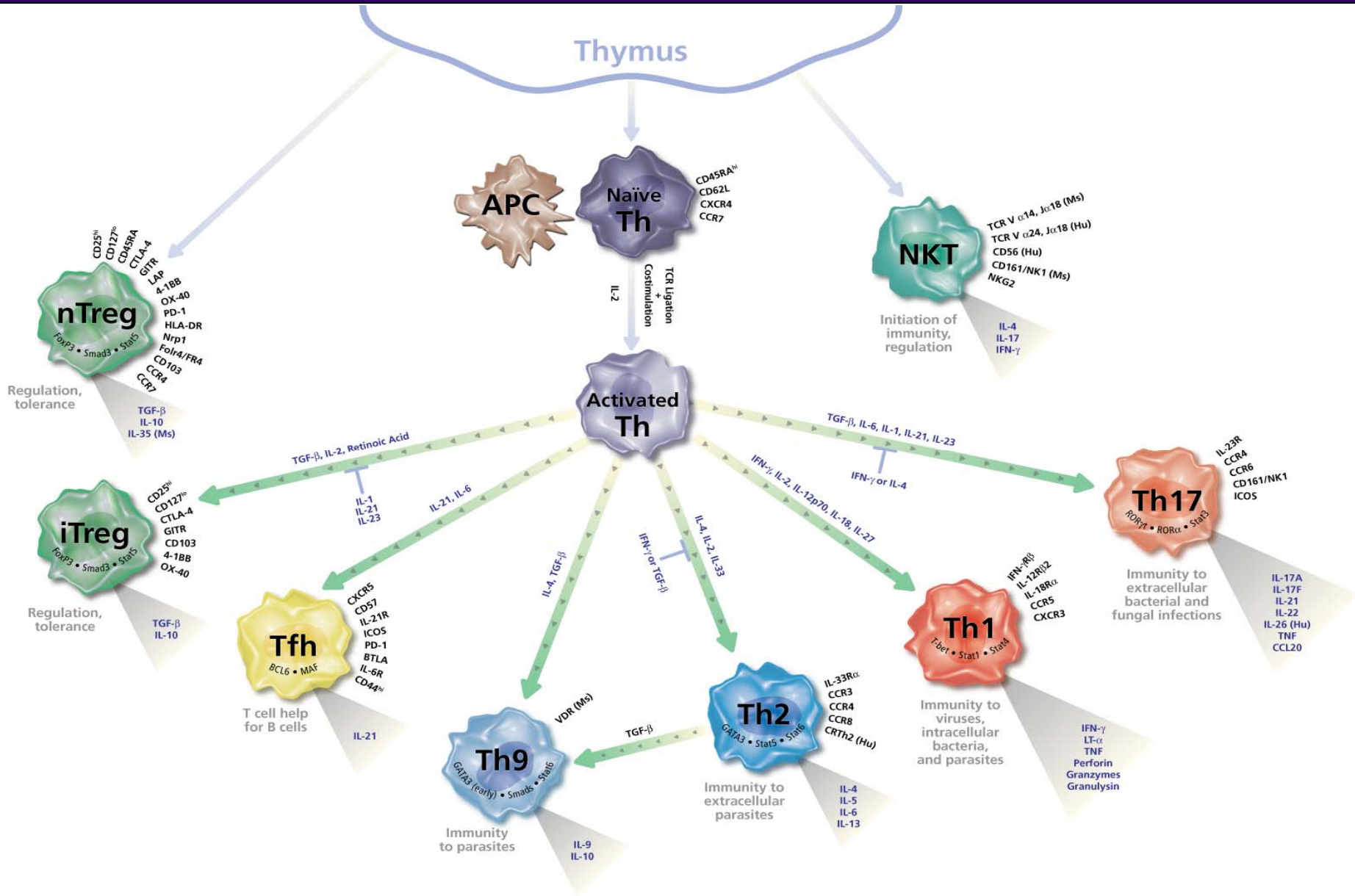
BD Biosciences

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)

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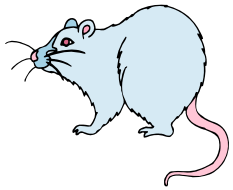
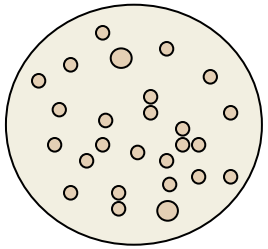
Experimental setup continued



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

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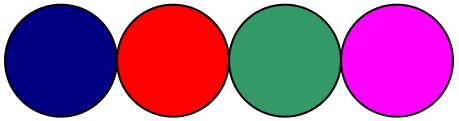
anti-CD28+IL-1β+IL-6+TGF-β+ IL-23



Day 1, 2, 3, 4

BD Cytofix/Cytoperm™ buffer
Cytokines

+ Monensin

Foxp3 Fix/Perm buffer
Foxp3 and some
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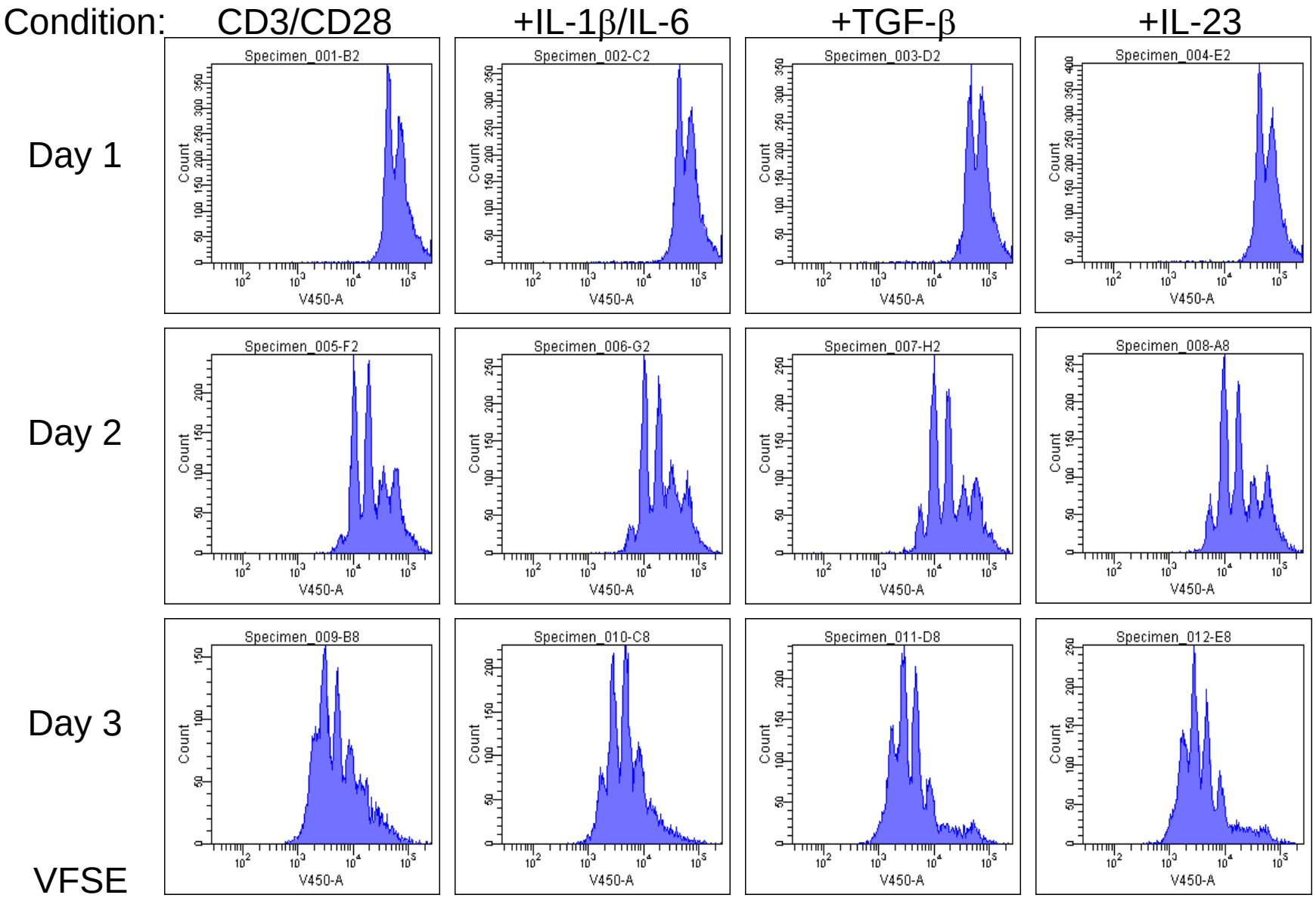
Harvest
Stimulate with PMA and
Ionomycin for 4–5 hours
+ or – Monensin

- Monensin

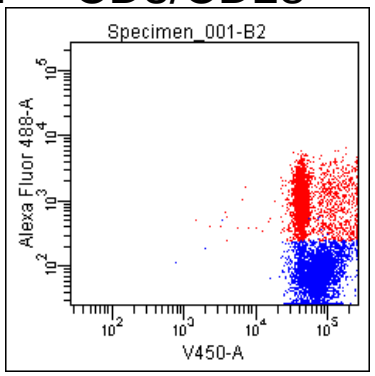
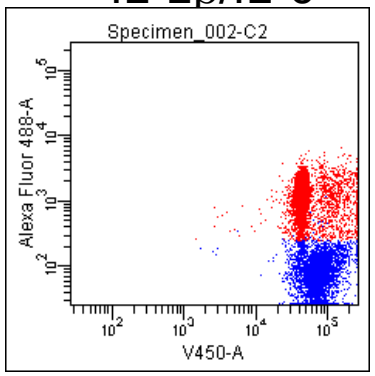
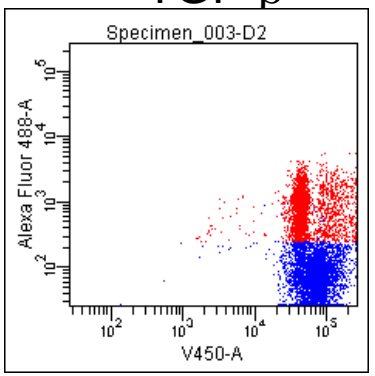
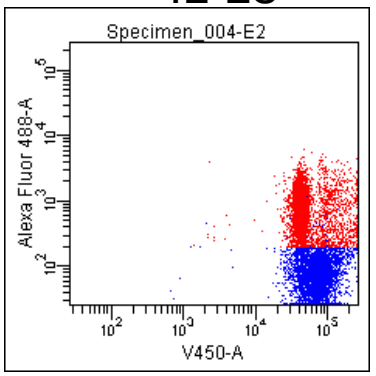
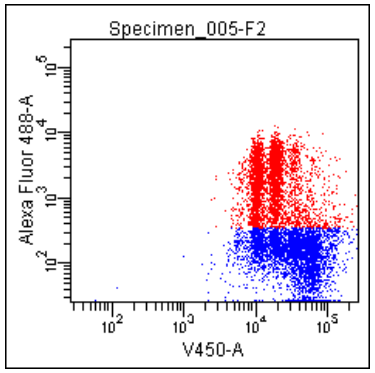
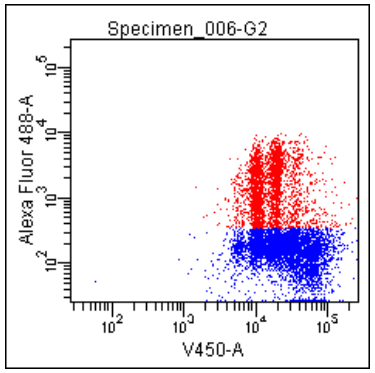
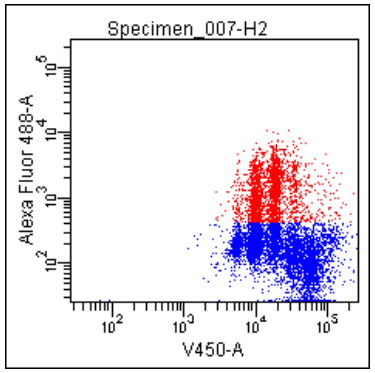
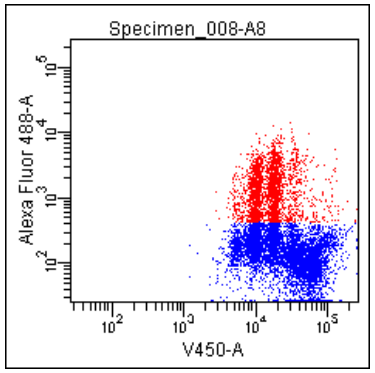
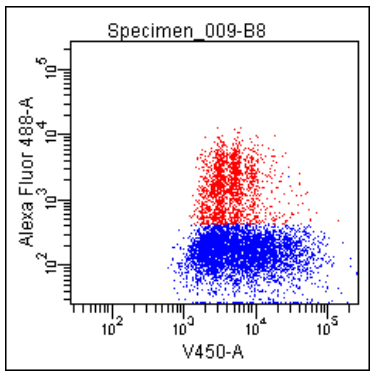
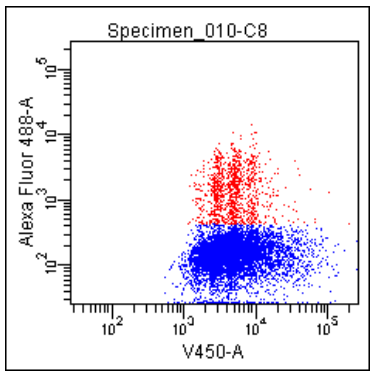
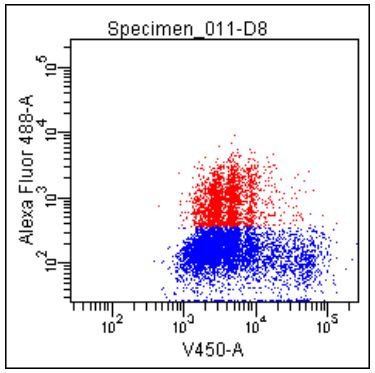
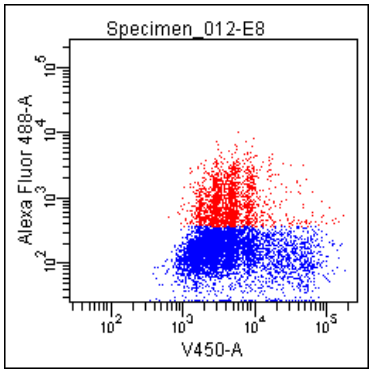
Supernatants: Analysis with BD™ CBA Flex Sets
Cells: BD™ Phosflow Fix and Perm buffer

BD

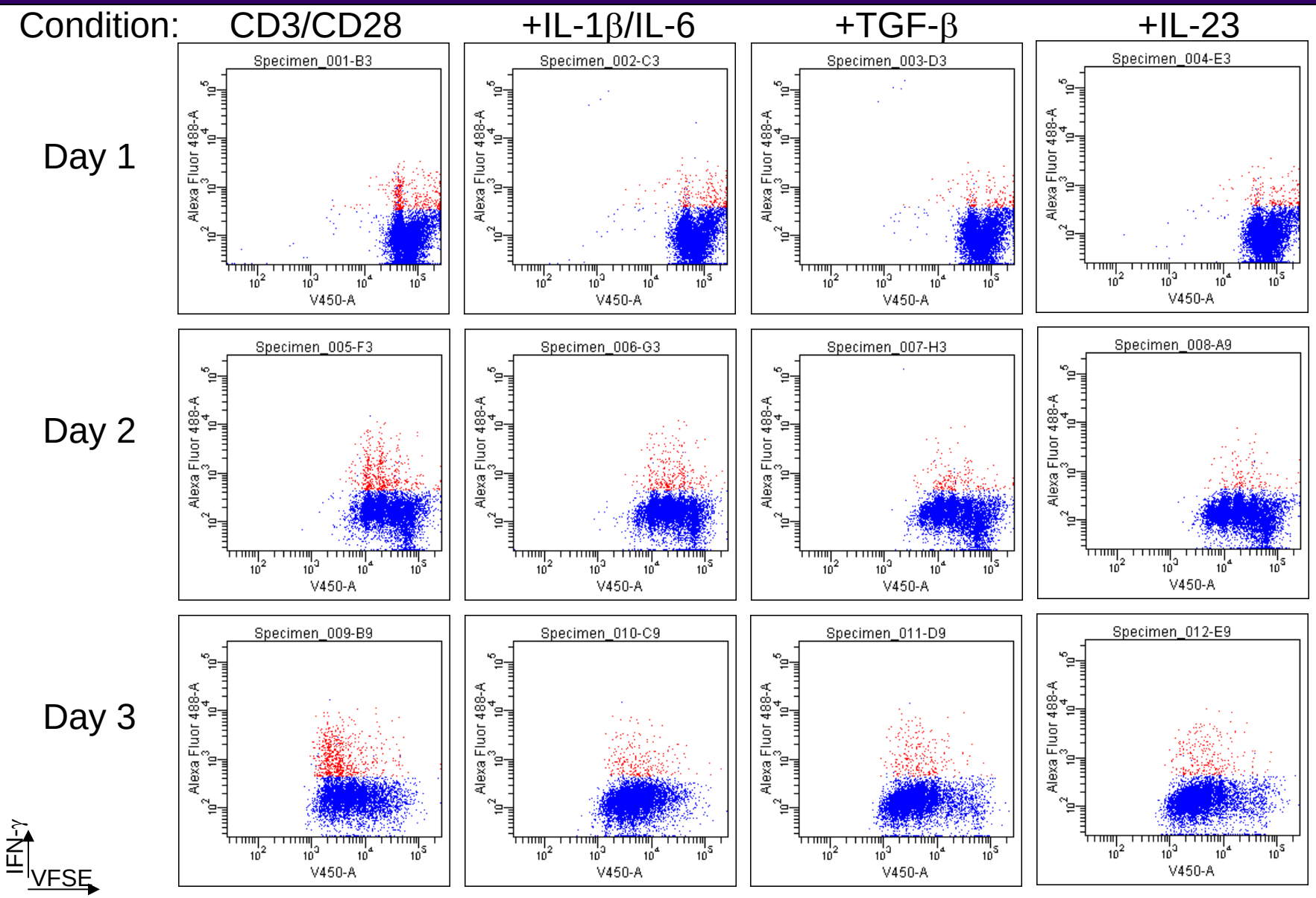
VFSE histograms



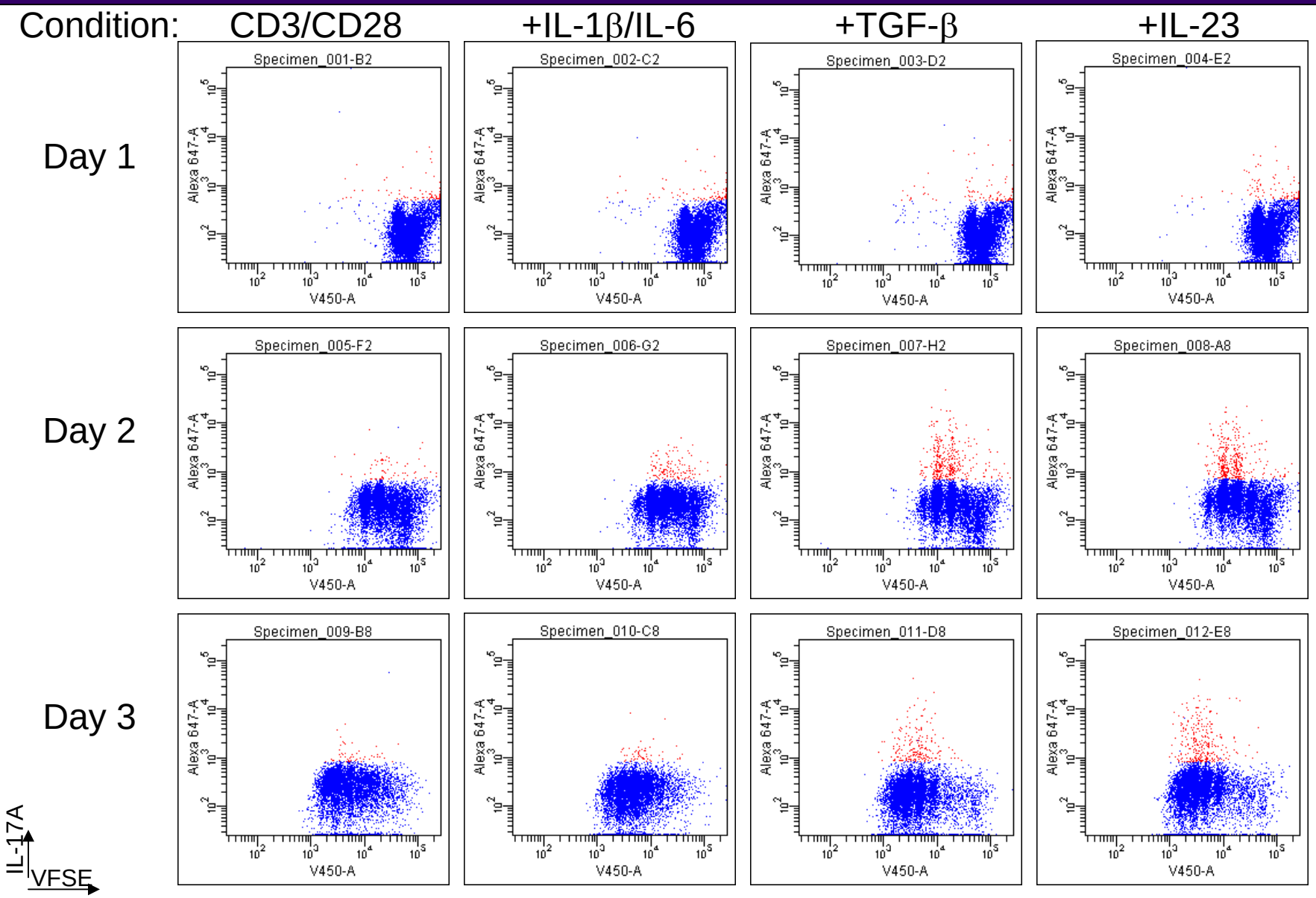
VFSE vs IL-2 data

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

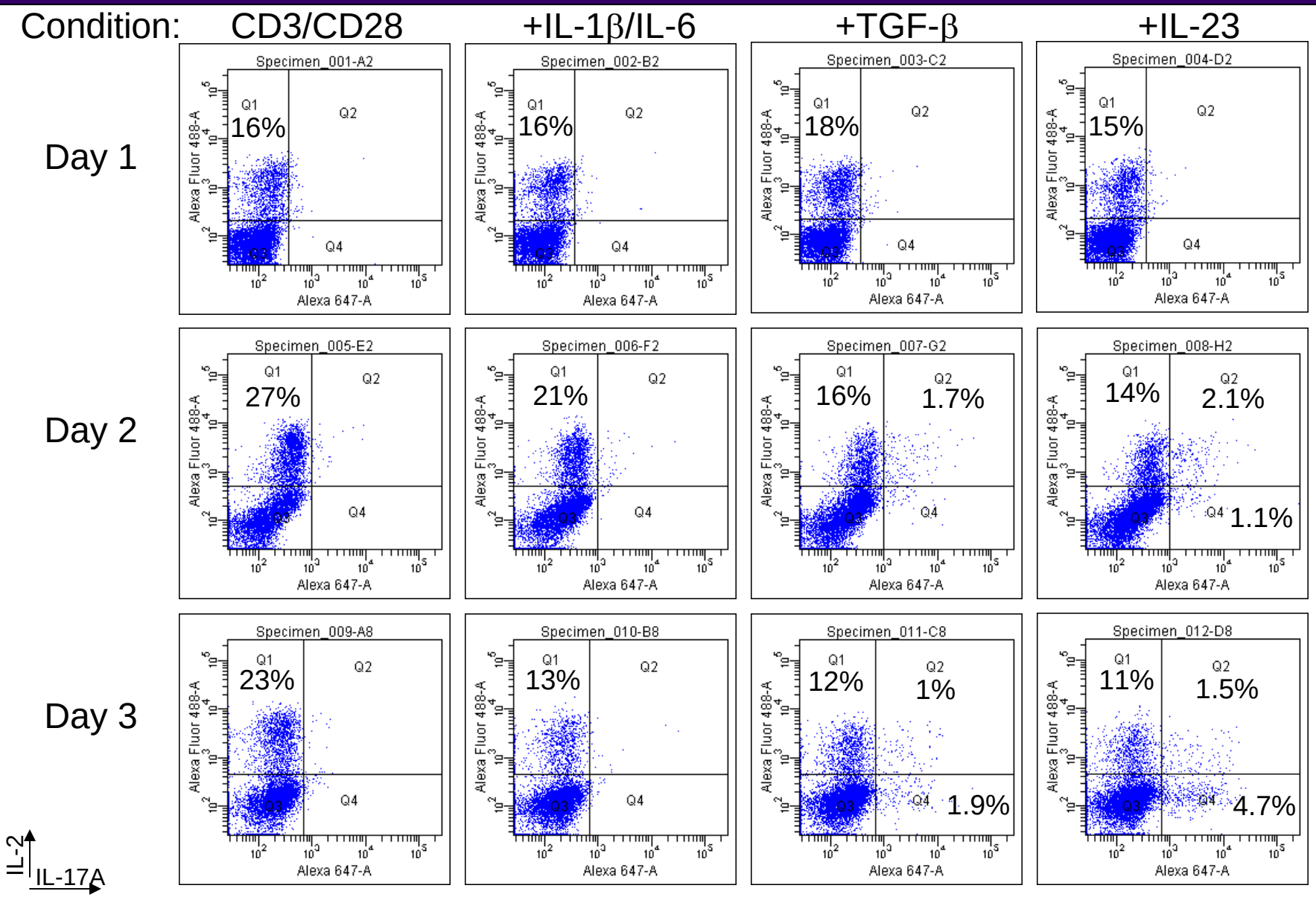
VFSE vs IFN-γ data



VFSE vs IL-17A data



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



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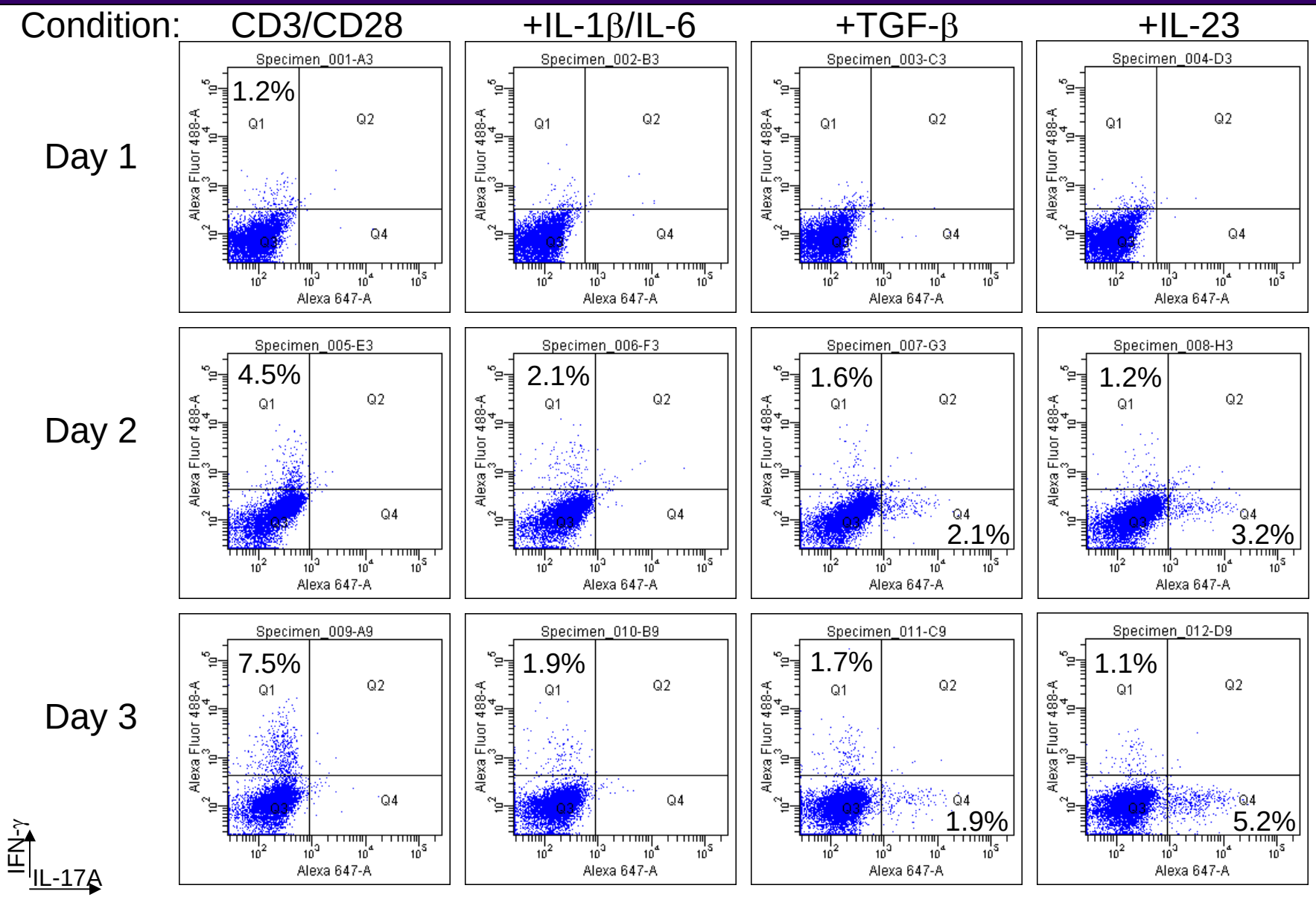
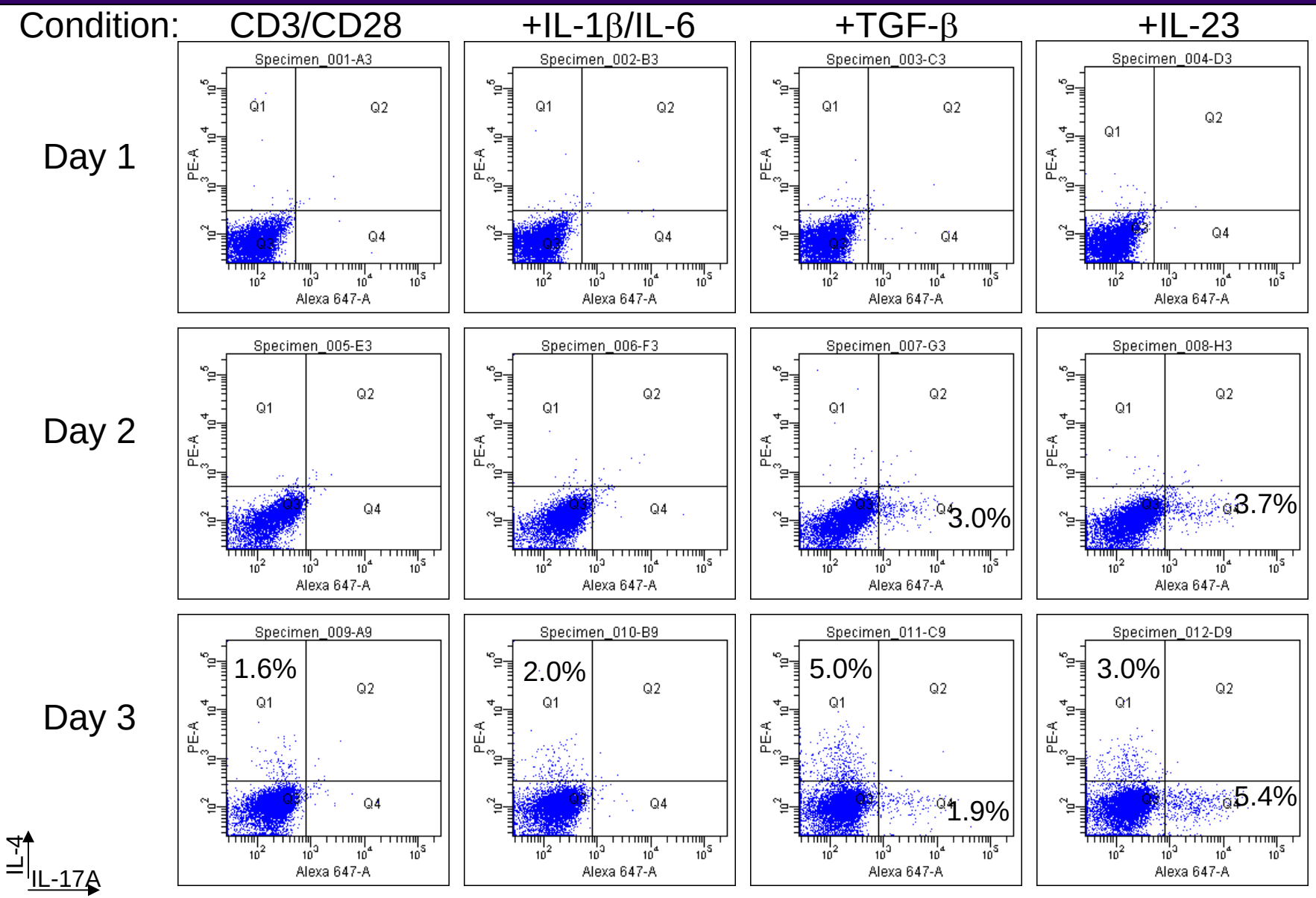
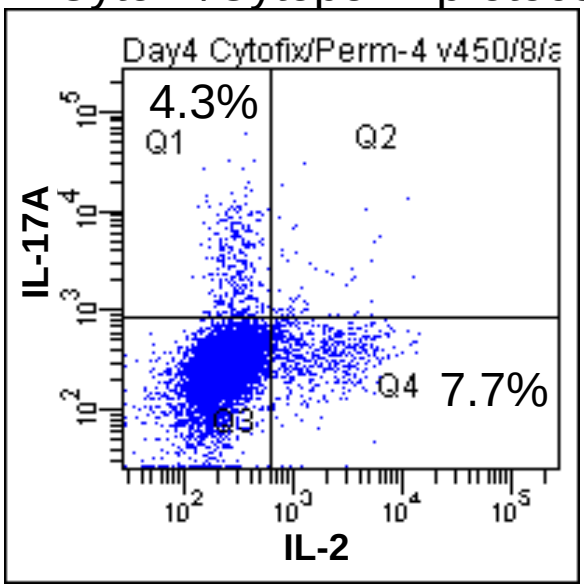


Co-expression of IL-17A vs IL-4

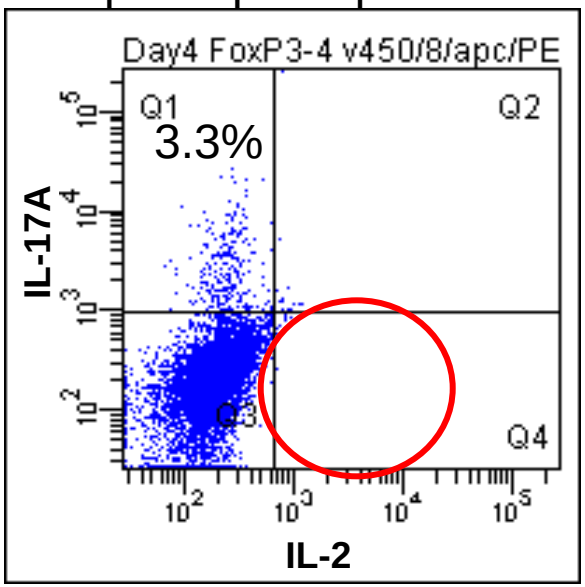


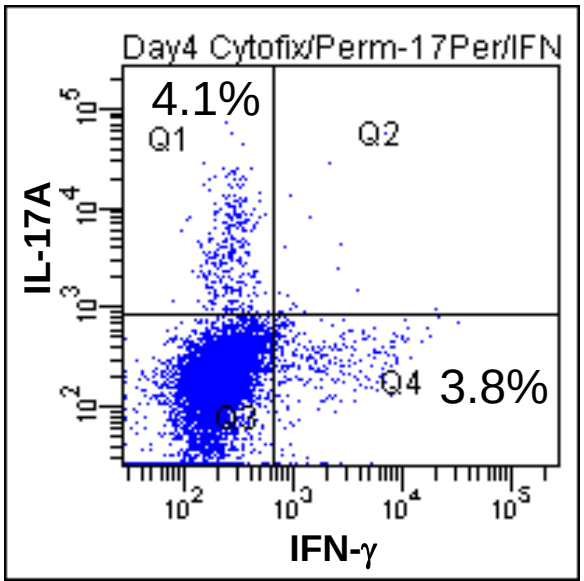
Comparison of two fix/perm protocols

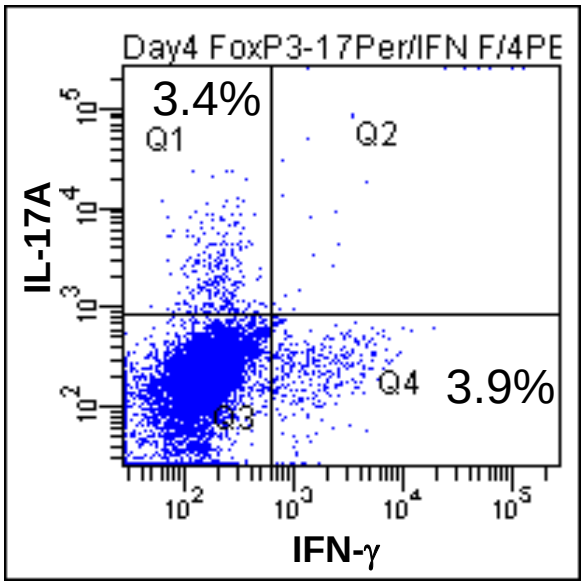
BD Cytofix/Cytoperm protocol



Foxp3 fix/perm protocol







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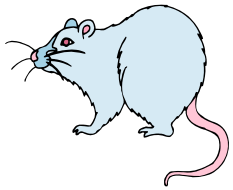

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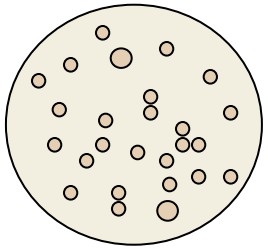

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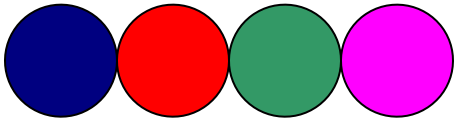

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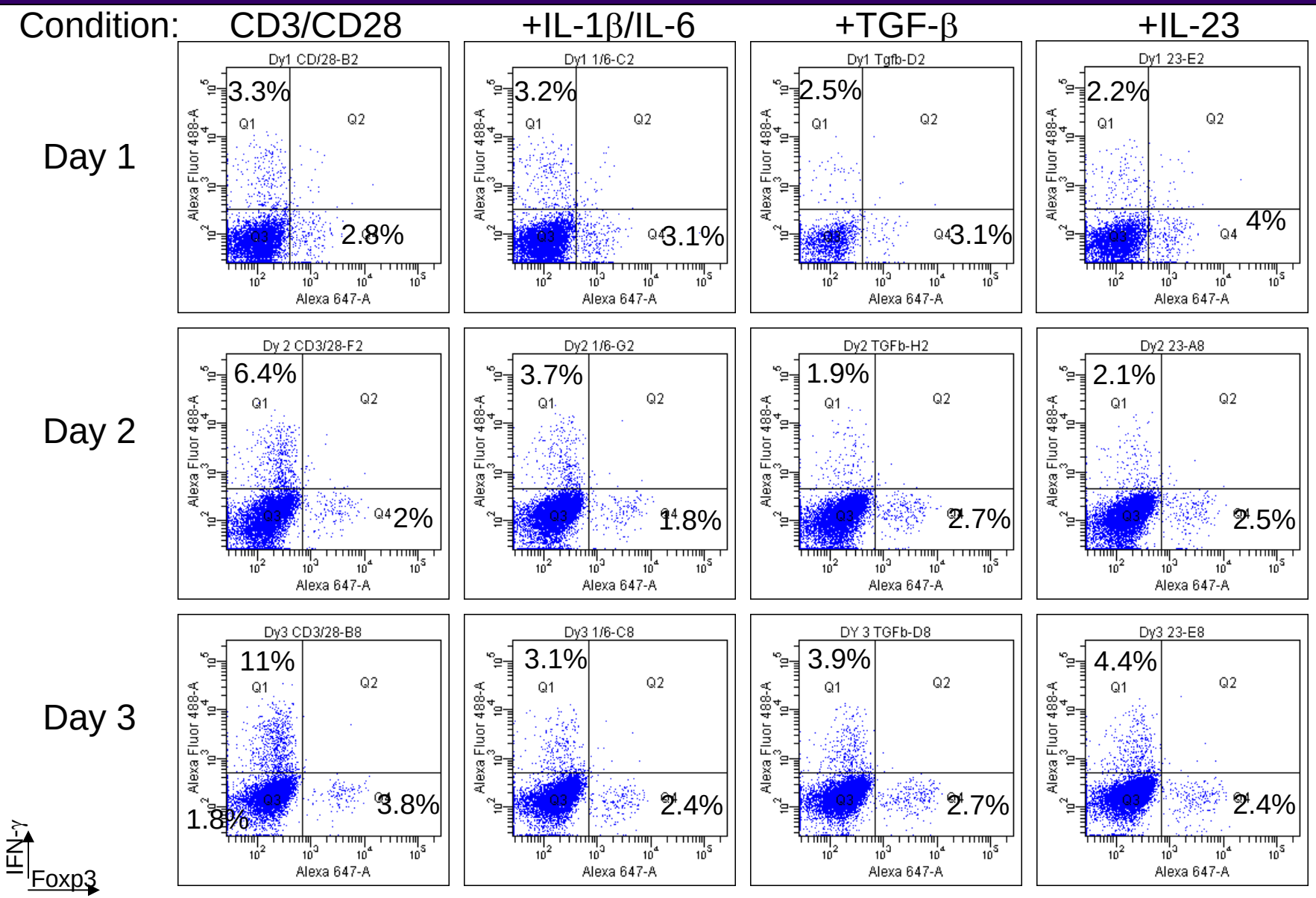

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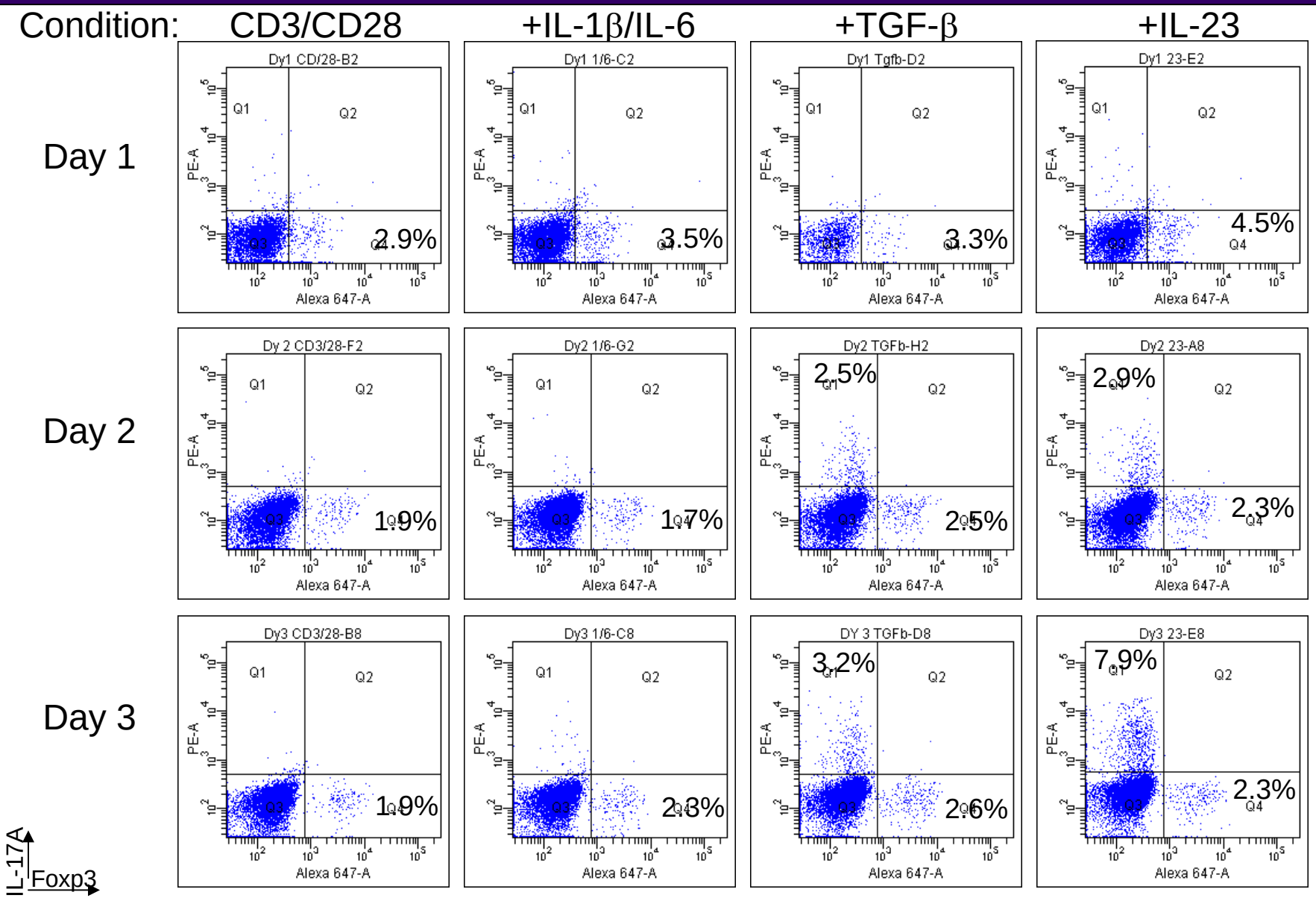
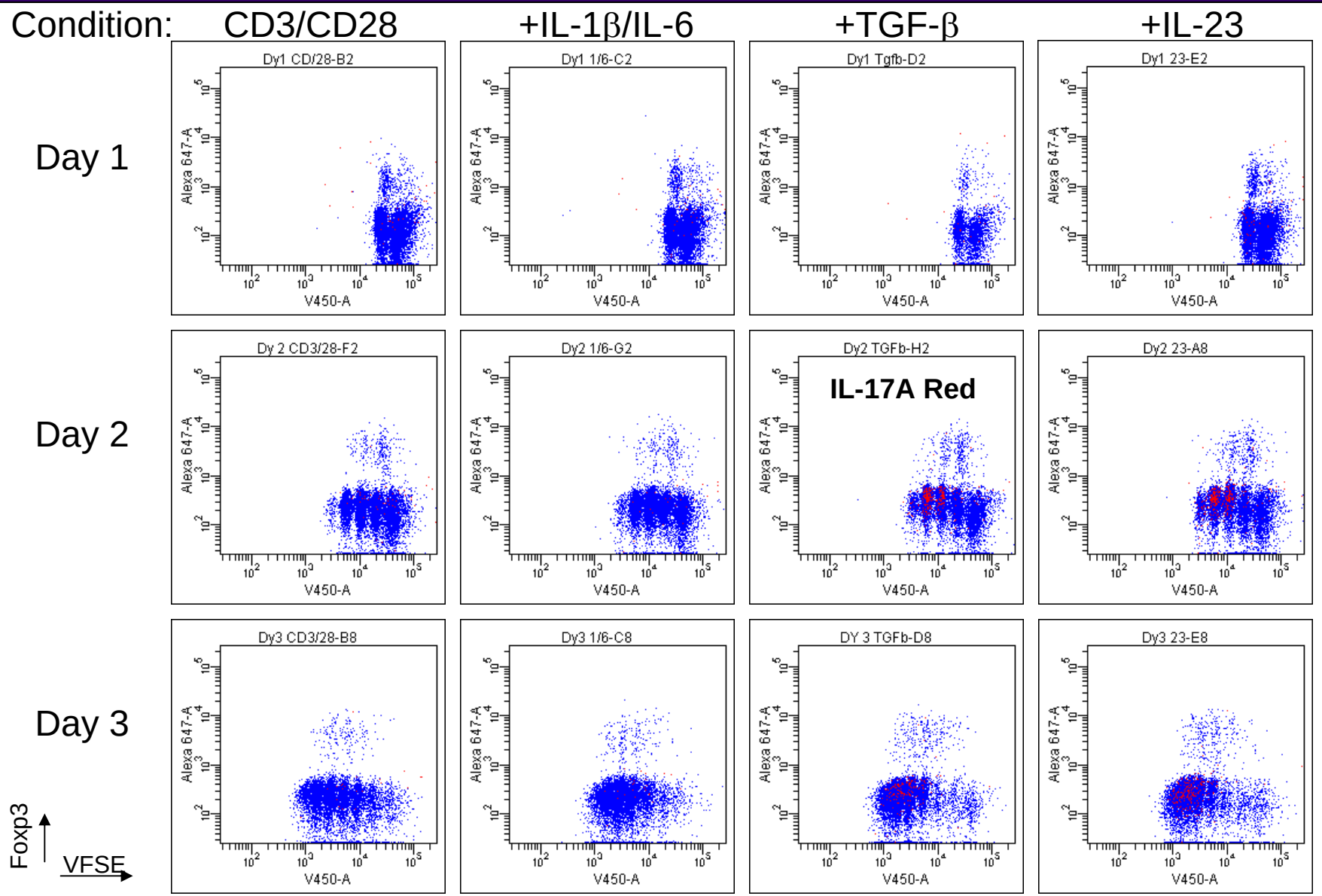


Proliferation of Treg and Th17 cells



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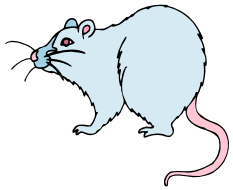
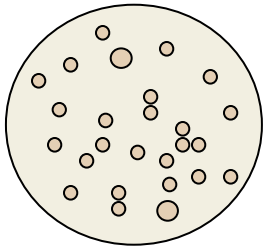


Harvest spleen



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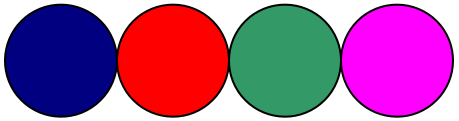

Day 1, 2, 3, 4

+ Monensin

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Foxp3 and some cytokines

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Stimulate with PMA and Ionomycin for 4–5 hours
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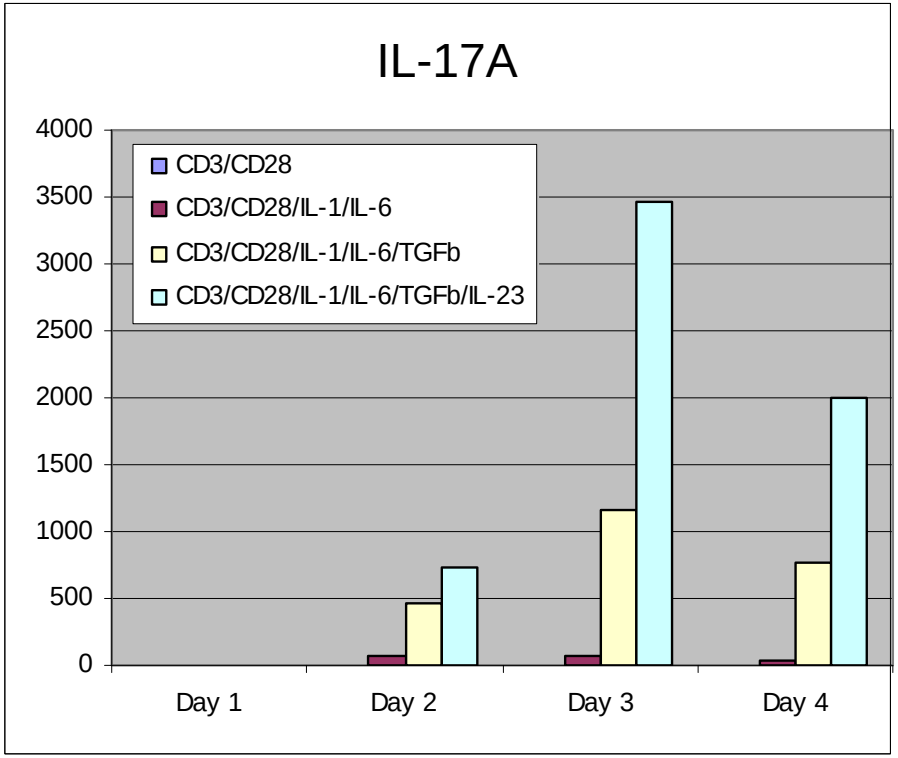
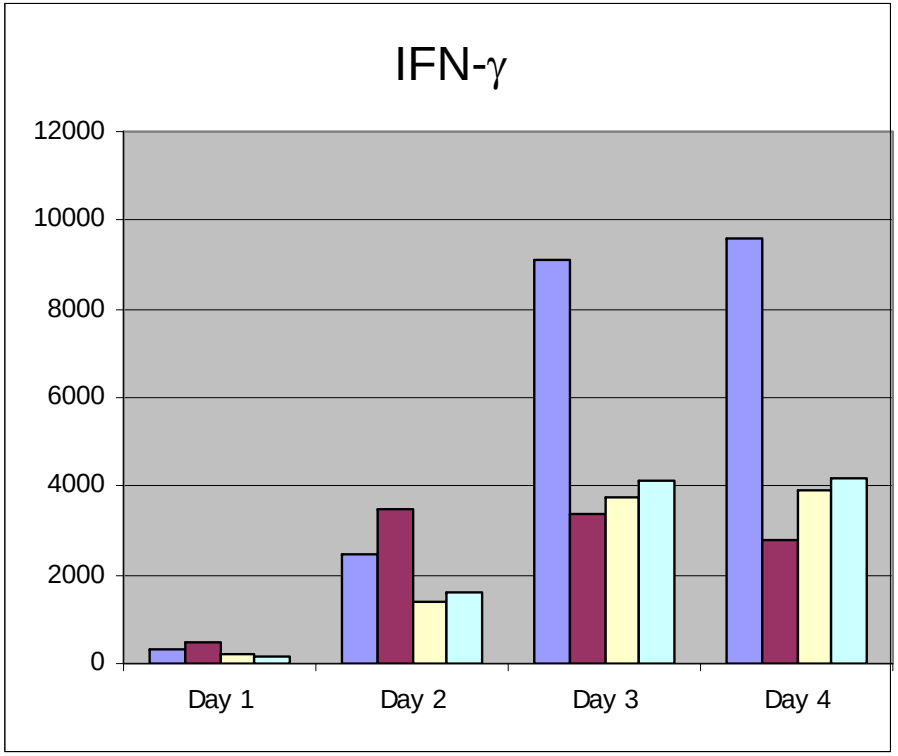
Cells: BD Phosflow Fix and Perm buffer

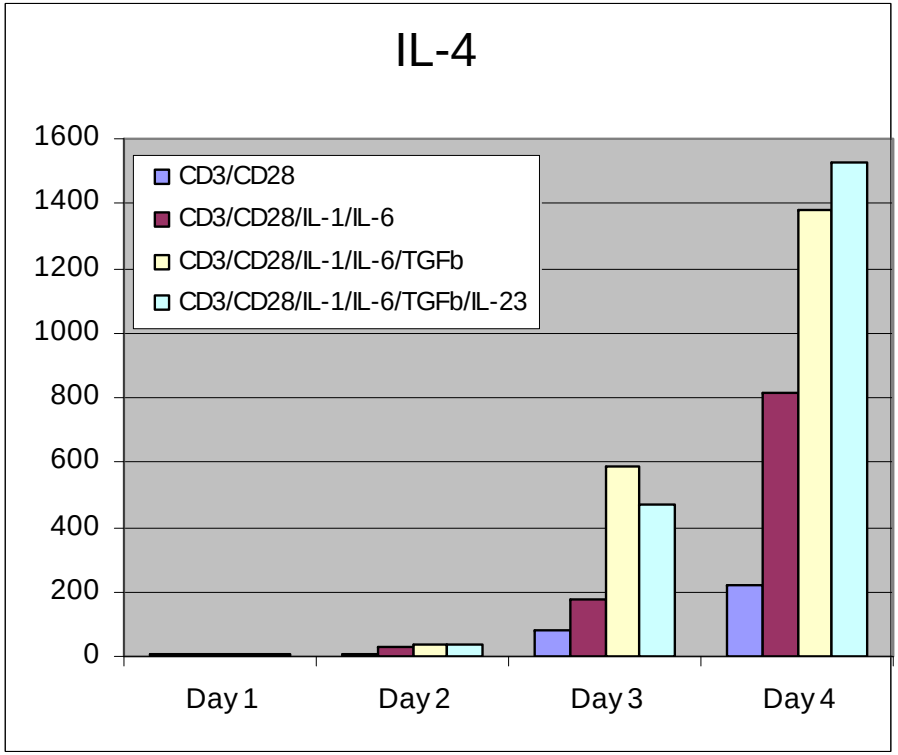
BD

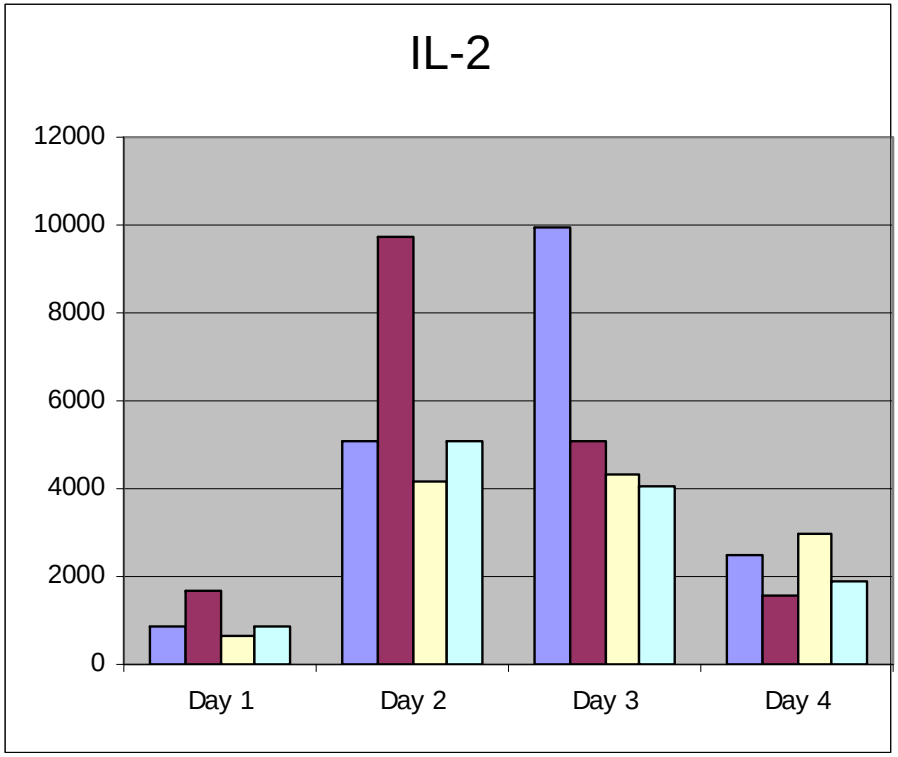
IL-17A and IFN-γ production





IL-4 and IL-2 production





BD

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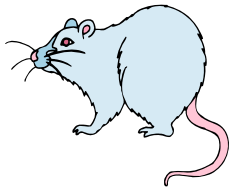

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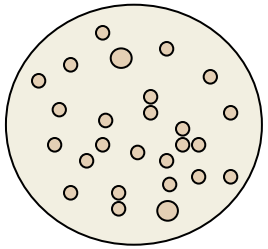

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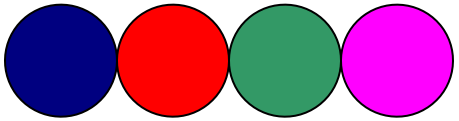

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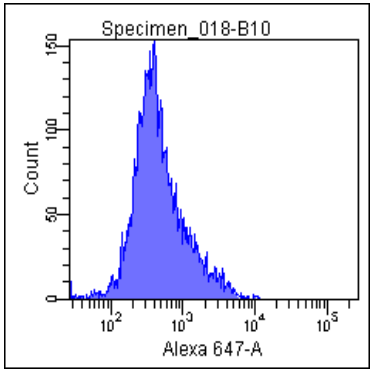
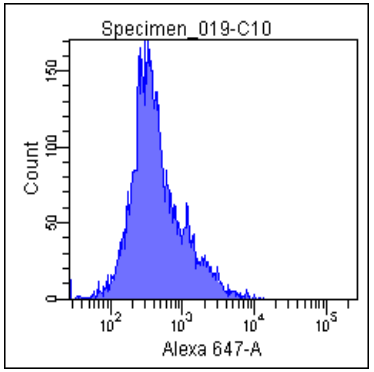
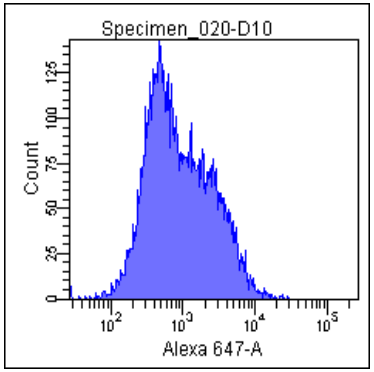
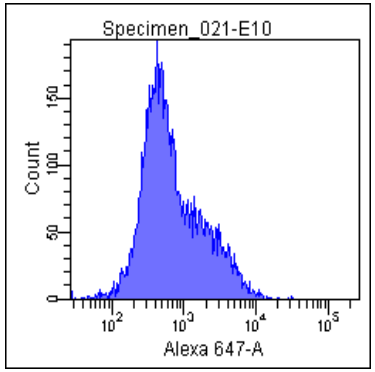
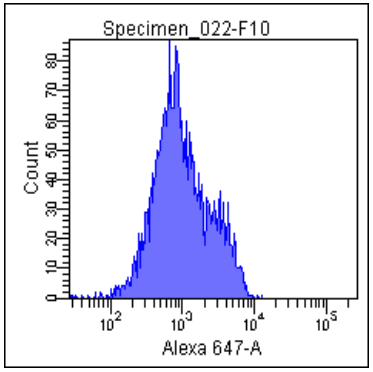
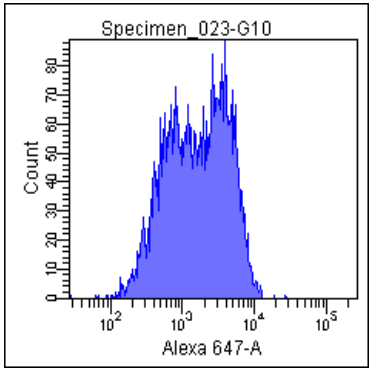
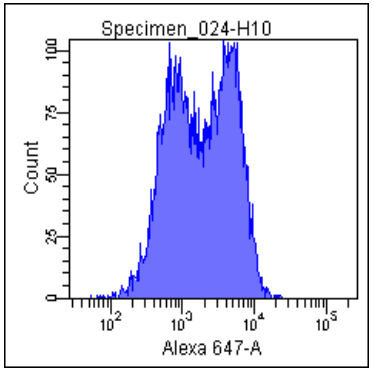
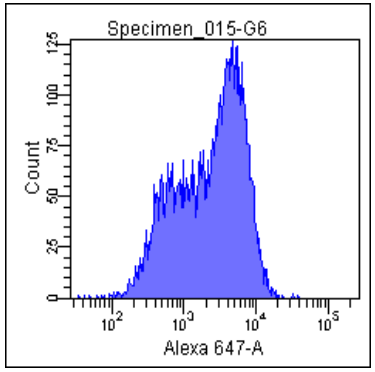
BD

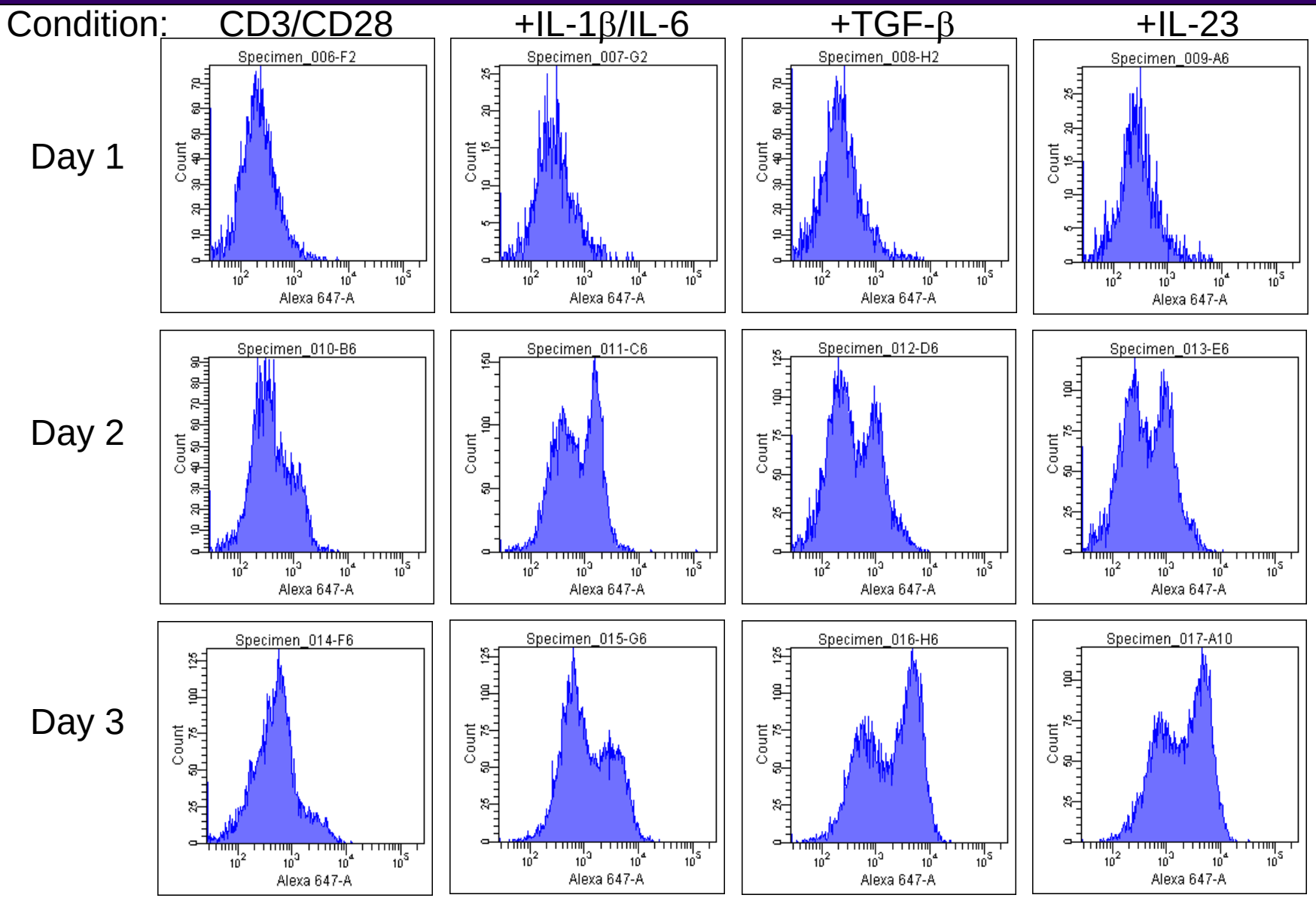
pStat5 detection on day 4

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

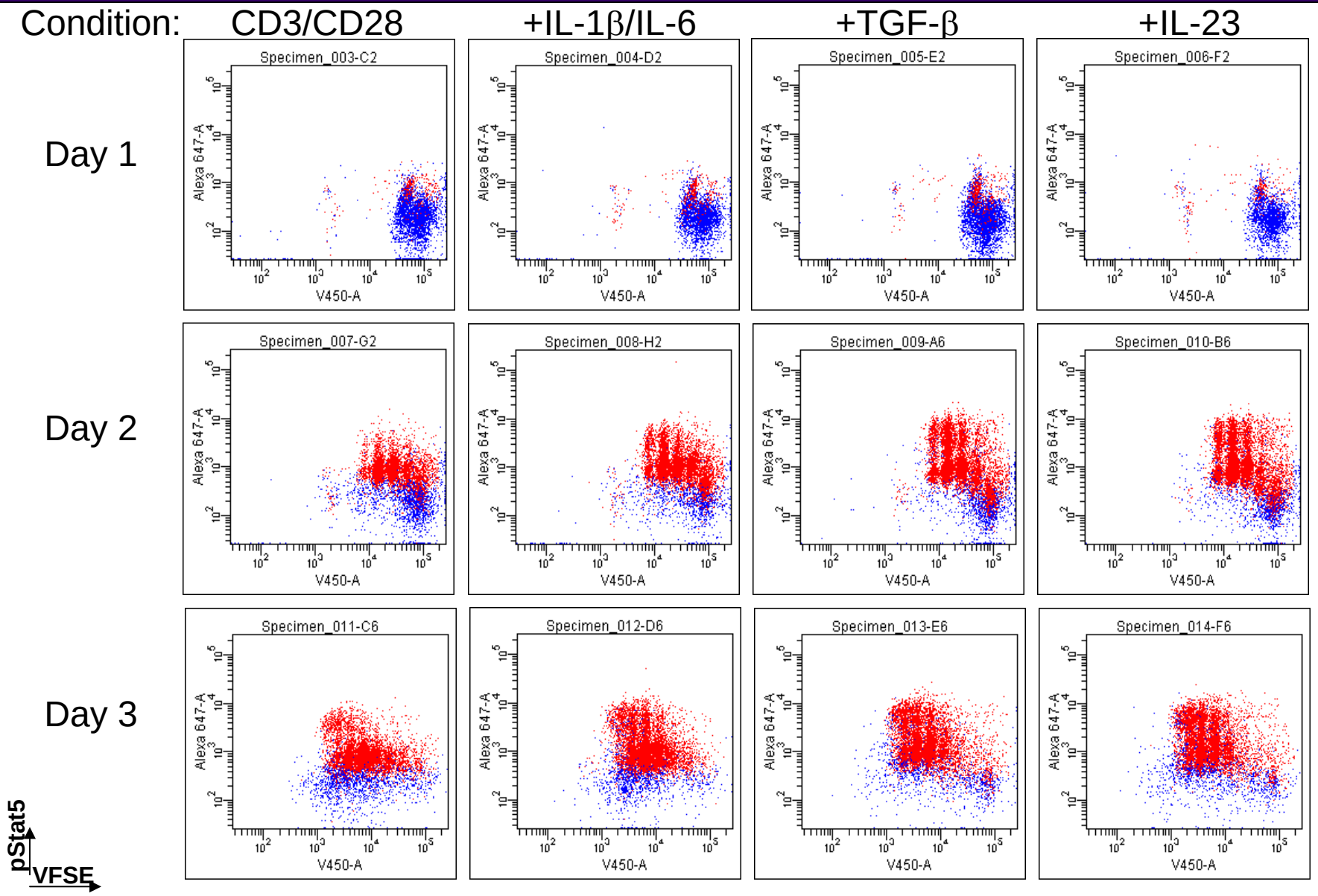
Activated: Cells were cultured and activated with PMA/Ionomycin for 5 hours and then stained with phosphospecific Stat5 antibody.

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

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