A Nanoscale Bead-Based Platform for the Generation of Melanoma-Specific CD8+ T Cells

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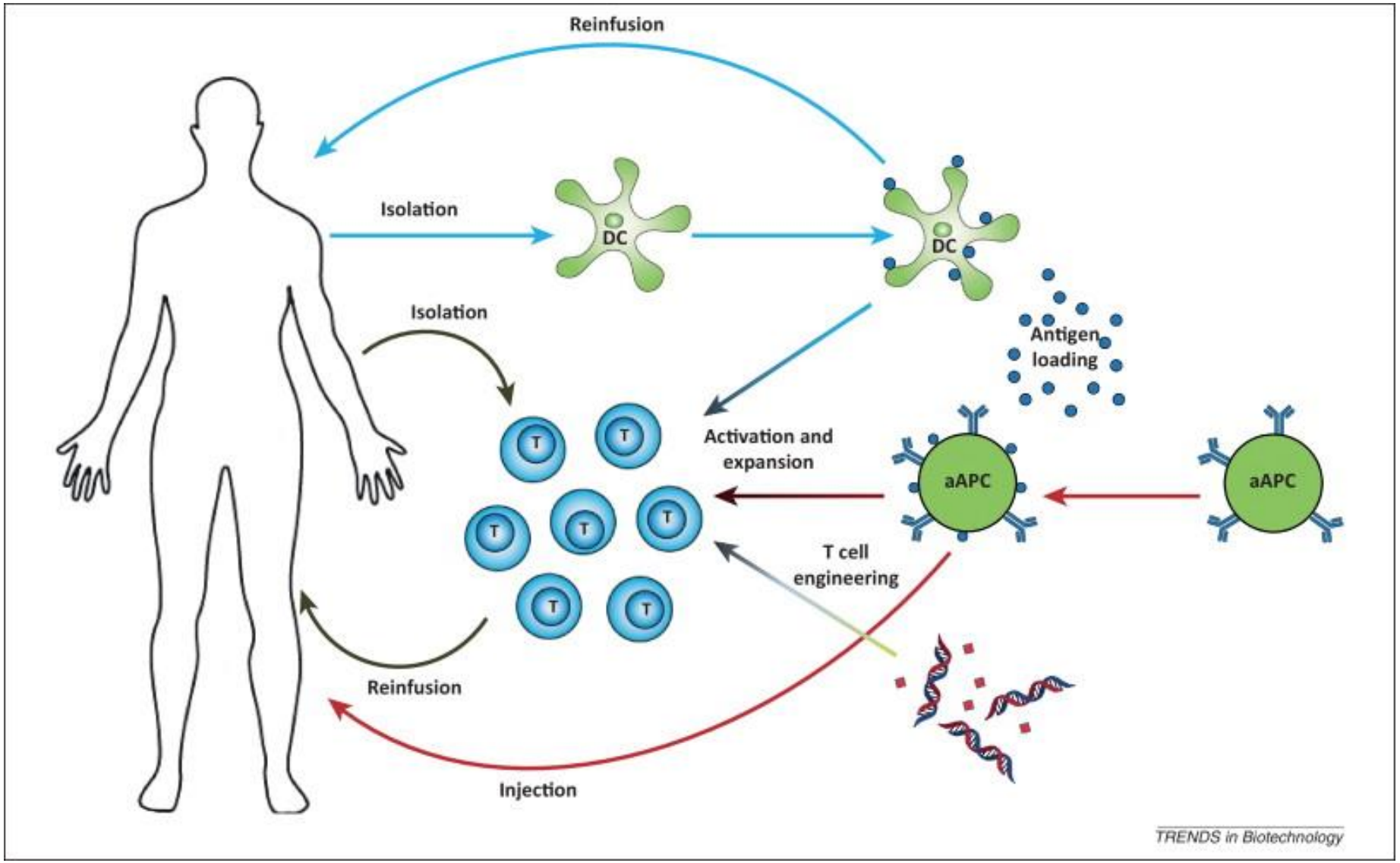
Immunotherapy Technologies



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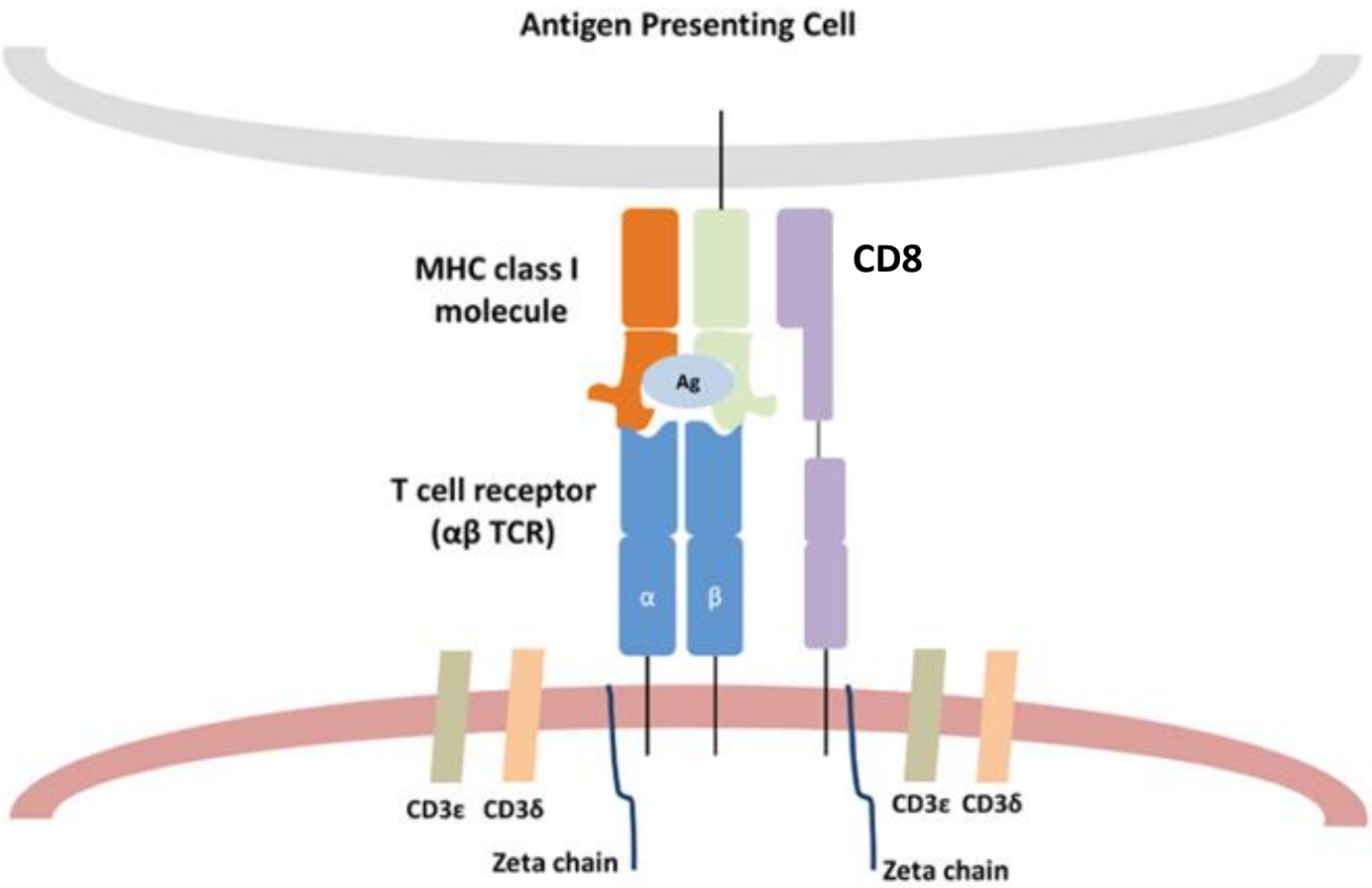
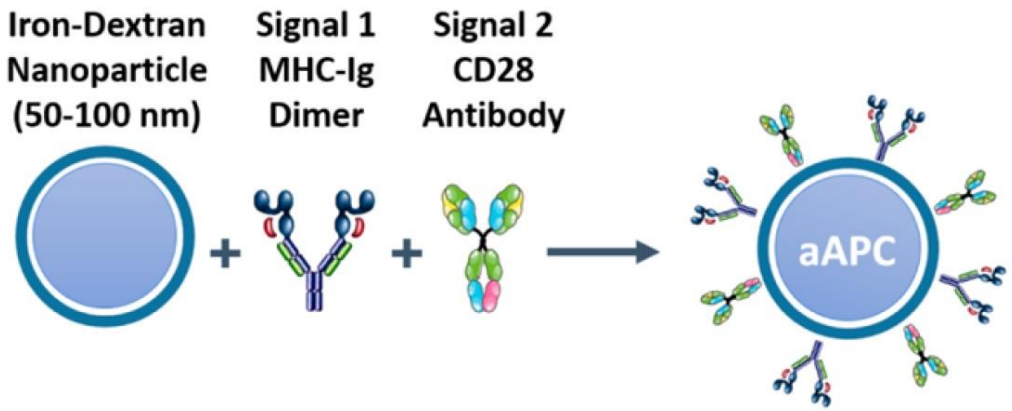
Antigen Presentation

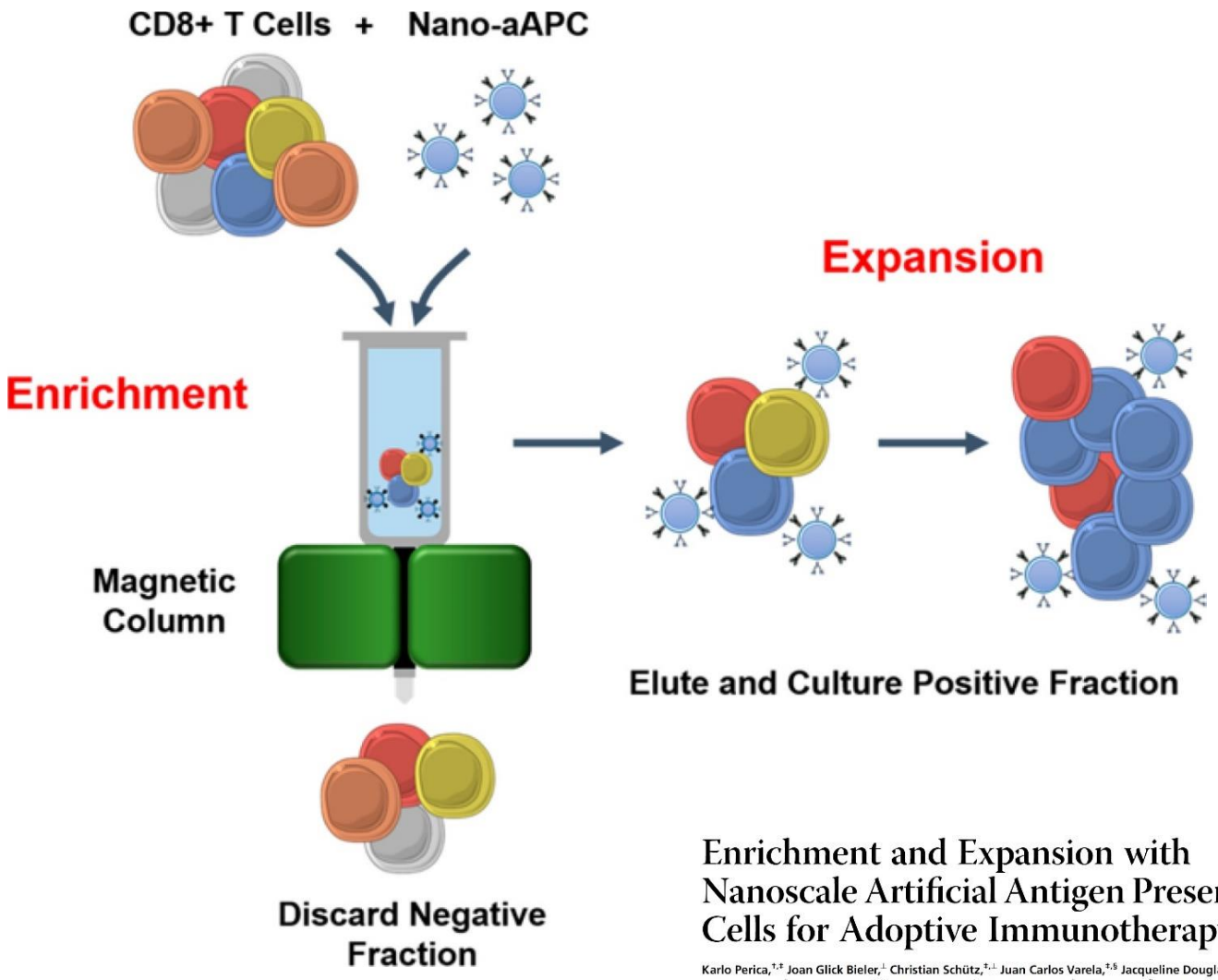


CD8+ T Cell

Adapted from Bio-Rad
An Overview of T Cell Receptors

Enrichment and Expansion





Enrichment and Expansion with Nanoscale Artificial Antigen Presenting Cells for Adoptive Immunotherapy

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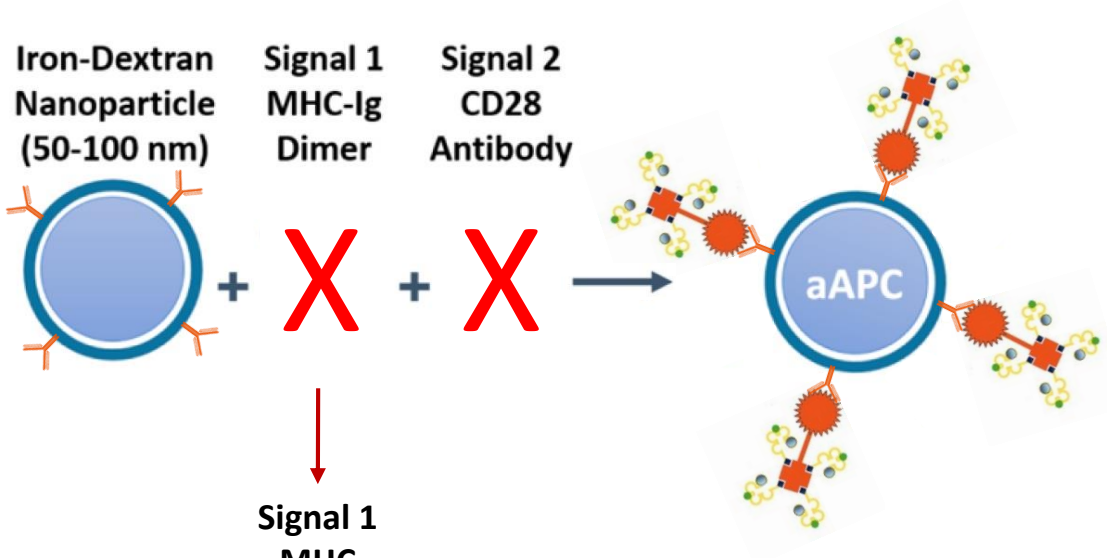
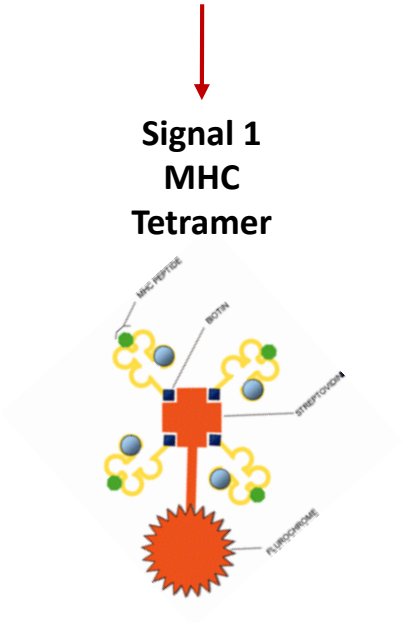
Signal 1 Only Tetramer Nanoparticle Design





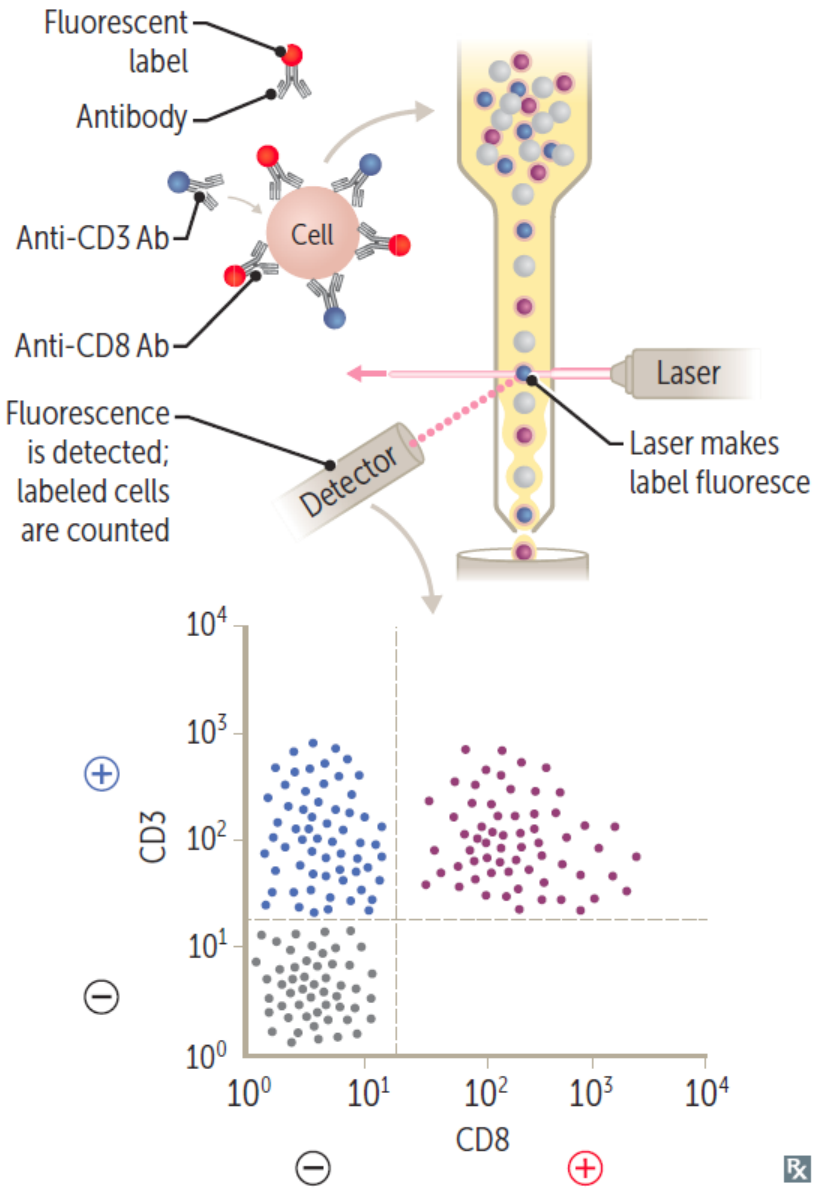
- Increase avidity
- Decrease non-specific interaction

MART-1 Pilot Experiment

- Healthy donor (80mL whole blood)
- Tetramer nanobead enrichment
- Soluble anti-CD28
- Conditioned media (cytokines)
- Re-stimulate every 7 days

Whole blood → PBMC Isolation → CD8+ Isolation → Enrichment + Expansion

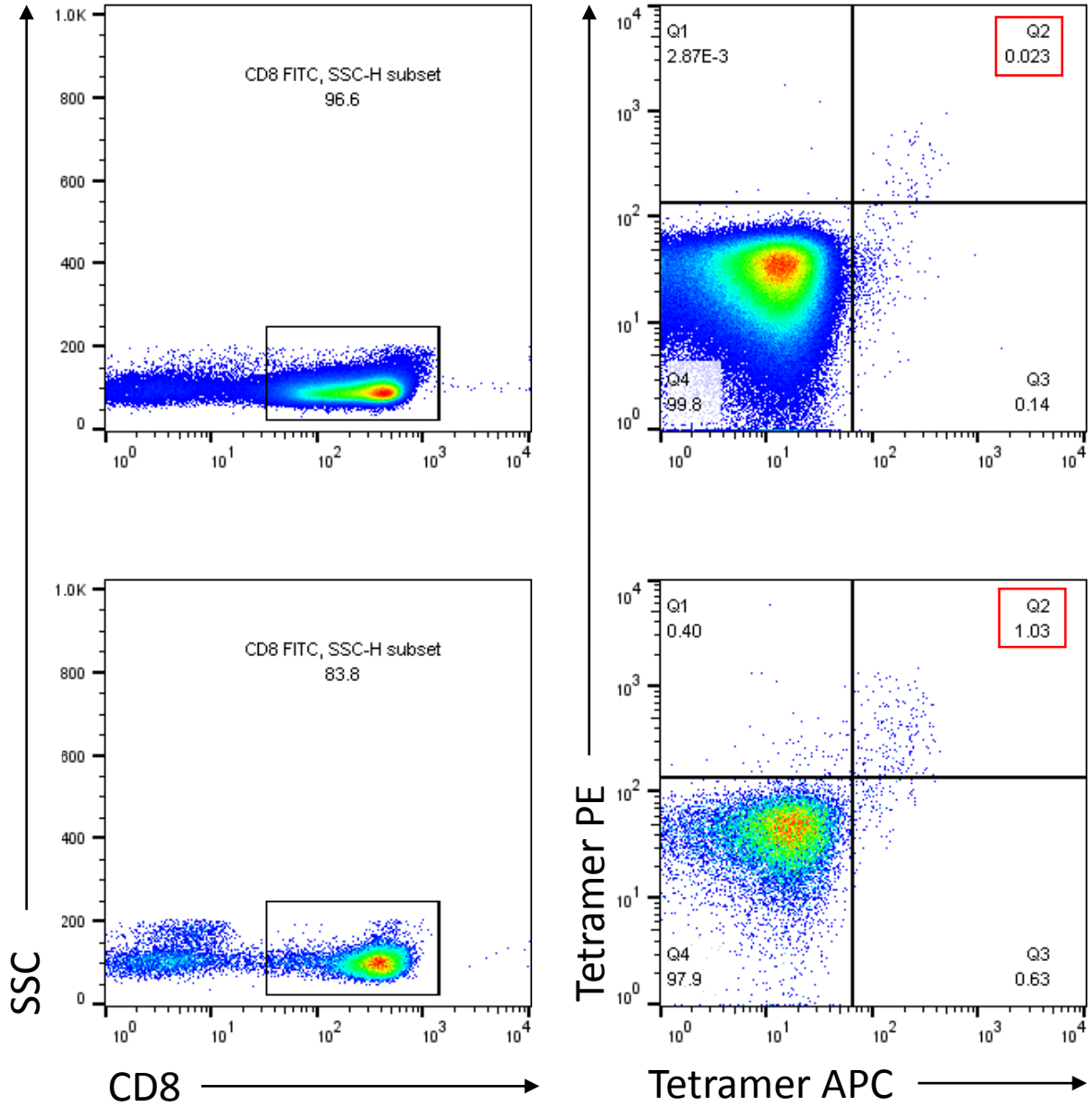
Flow Cytometry



Day 0
MART-1

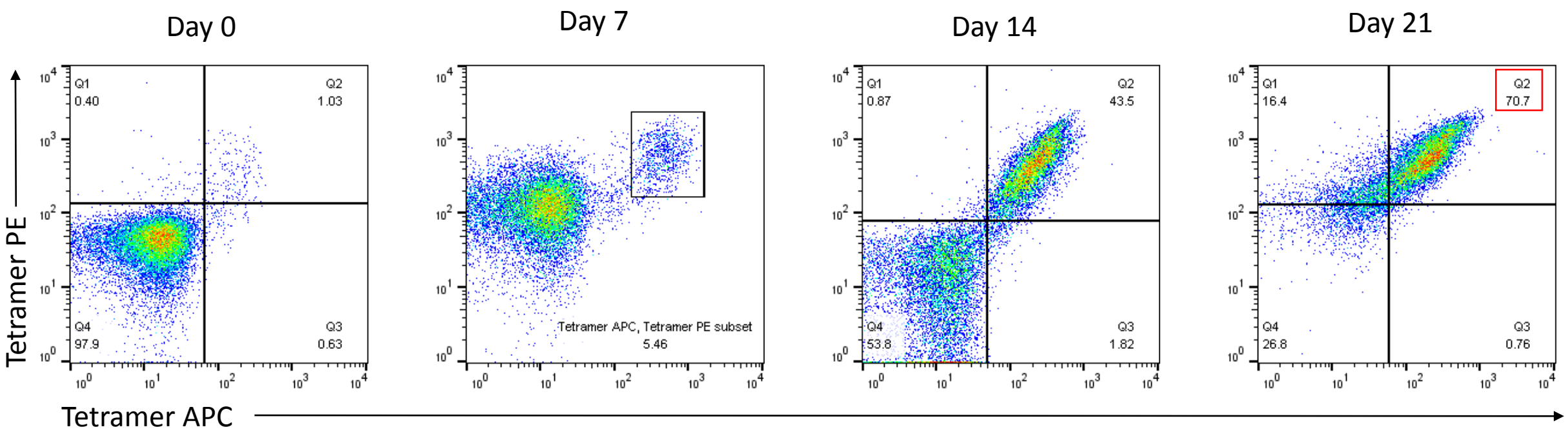
Pre-Enrichment

Post-Enrichment

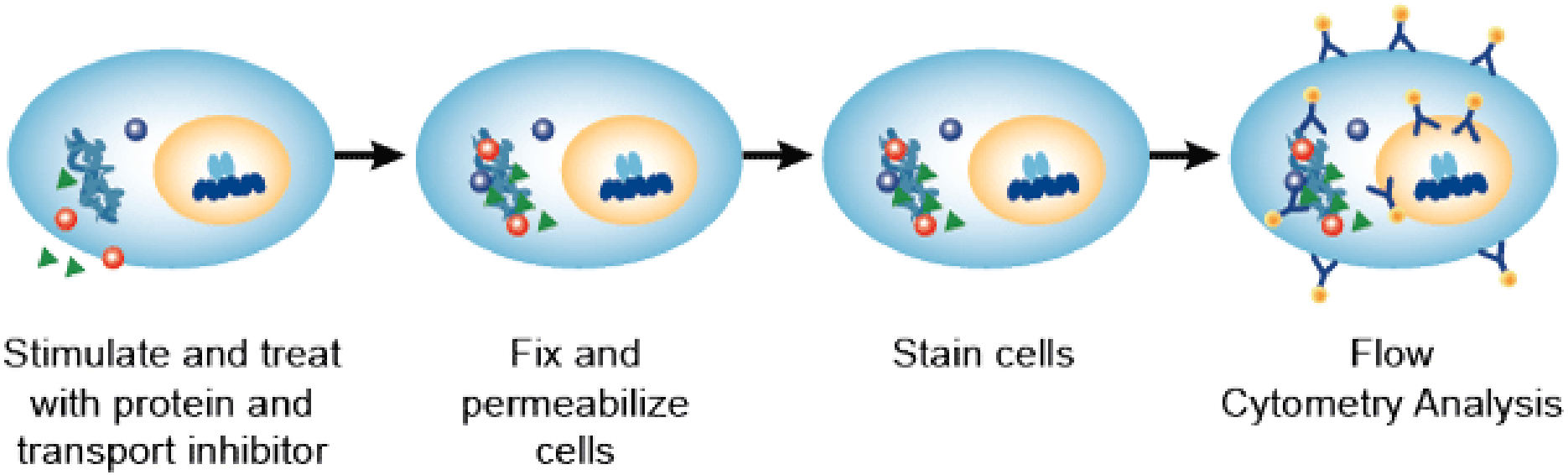


MART-1
Bead-Based Signal 1
Soluble Signal 2

Are they functional?



Intracellular Cytokine Staining

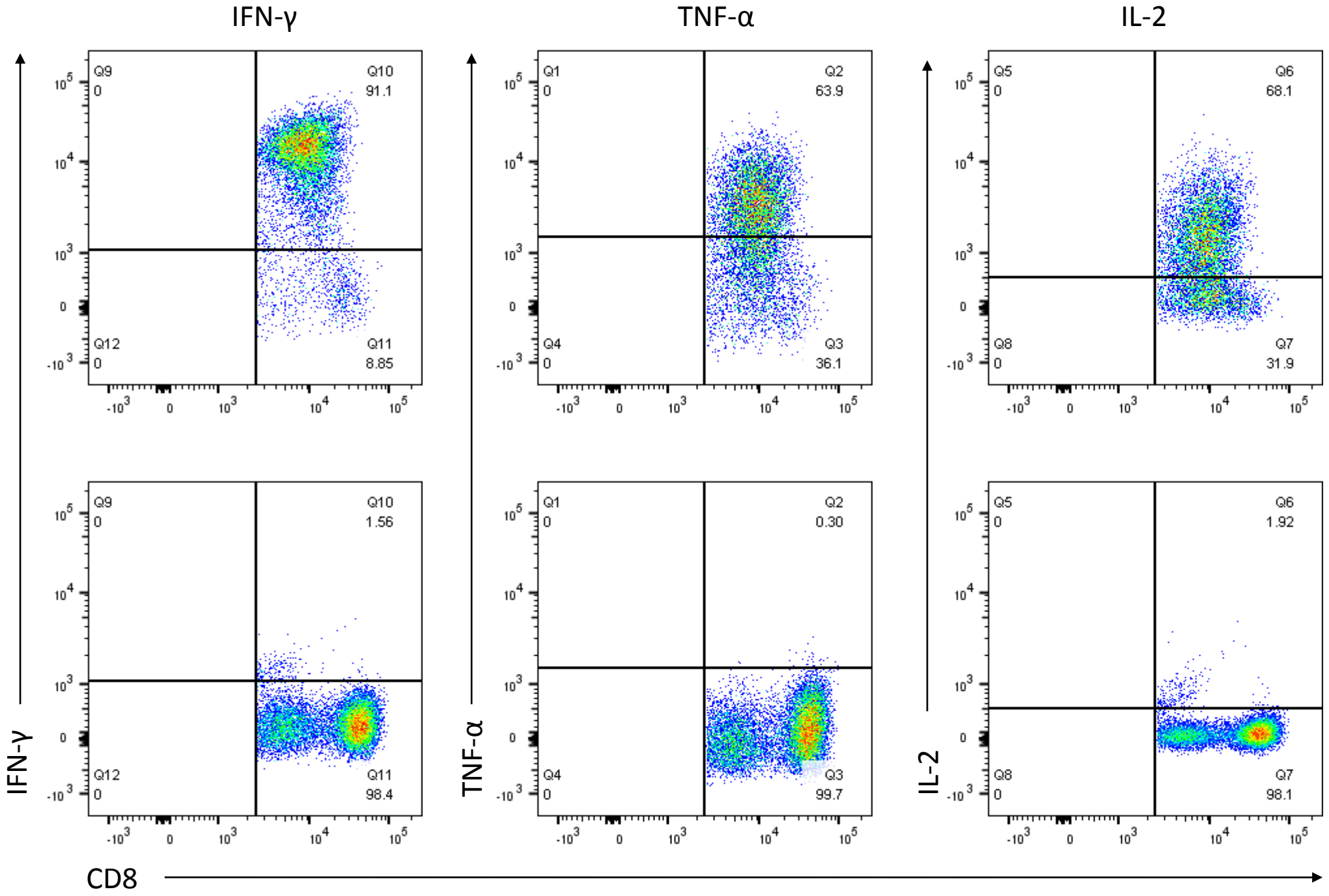


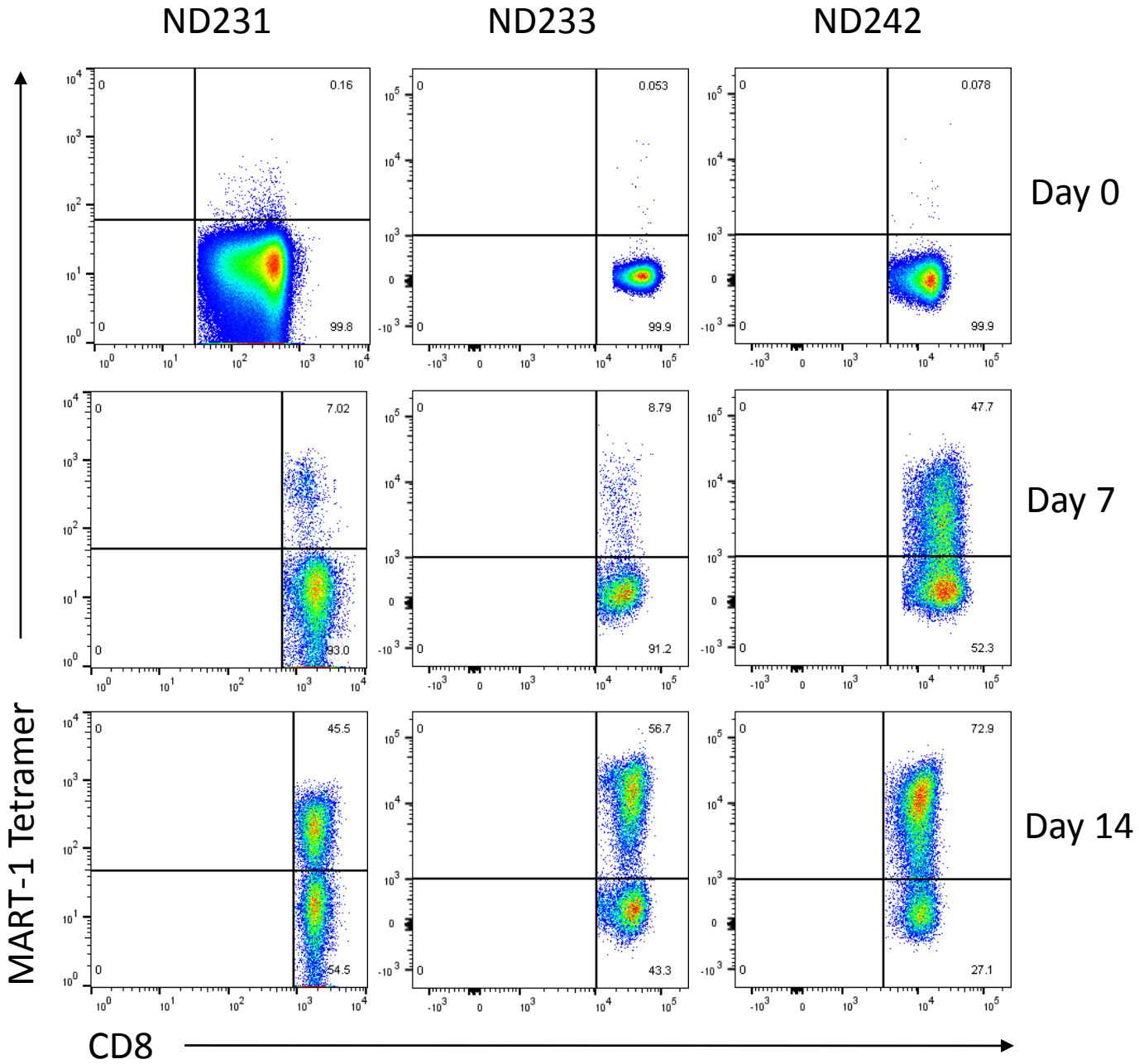
http://www.immunecarta.com/en/immune-monitoring-services-technology/functional-profiling.html

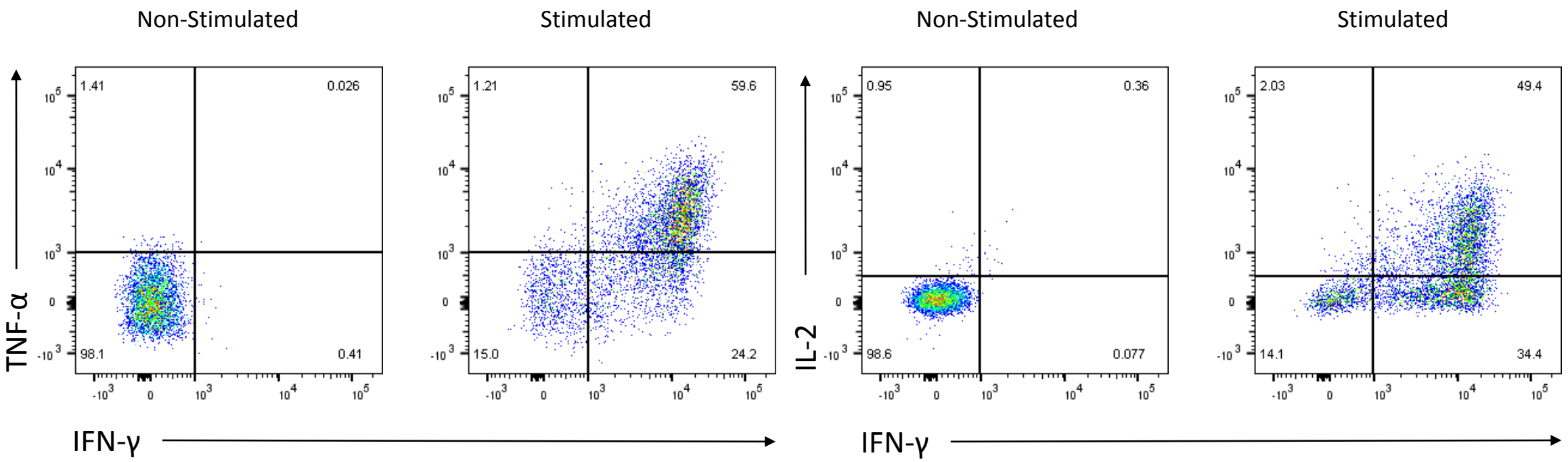
Day 21
ICS Assay

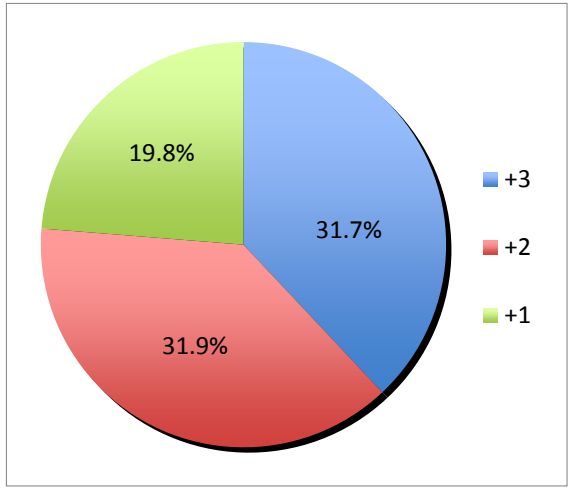
T2 + MART-1

T2 Control



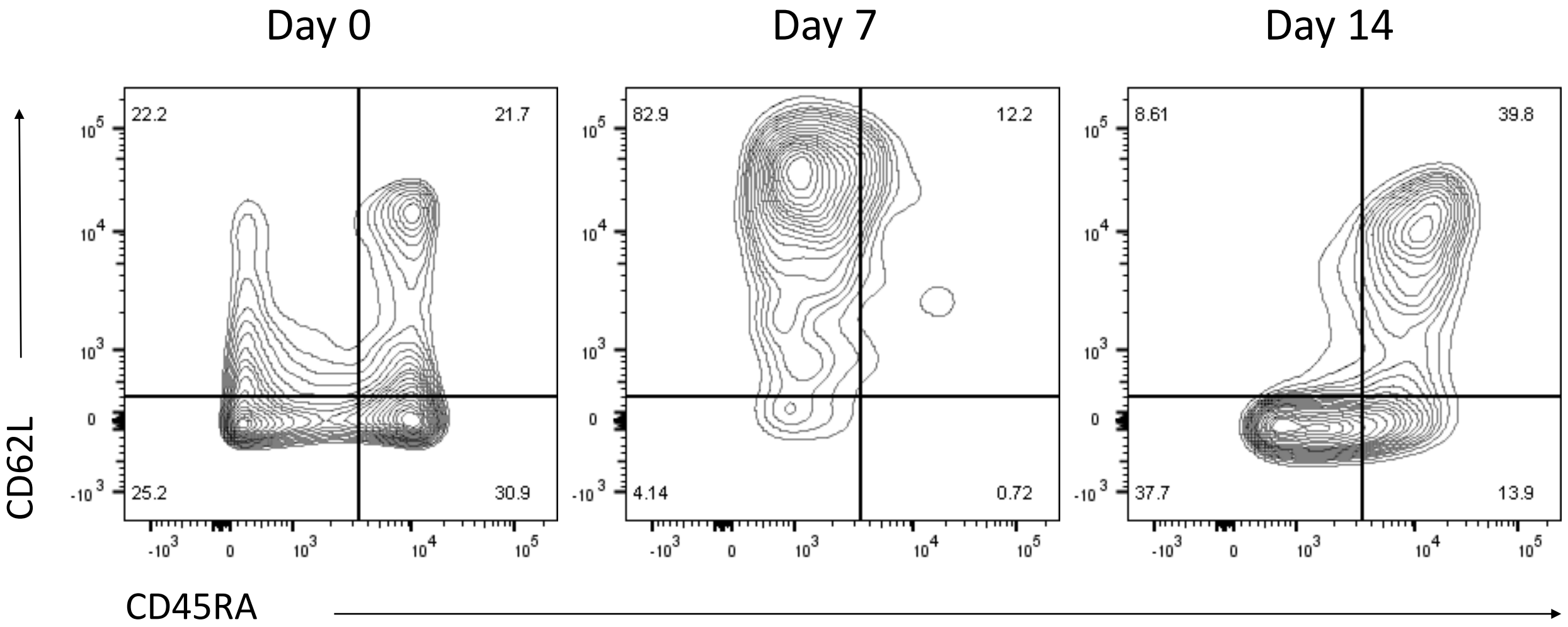

ND231
ND233
ND242
Day 0
Day 7
Day 14
MART-1 Tetramer
CD8
0.16
99.8
0.053
99.9
0.078
99.9
7.02
93.0
8.79
91.2
47.7
52.3
45.5
54.5
56.7
43.3
72.9
27.1





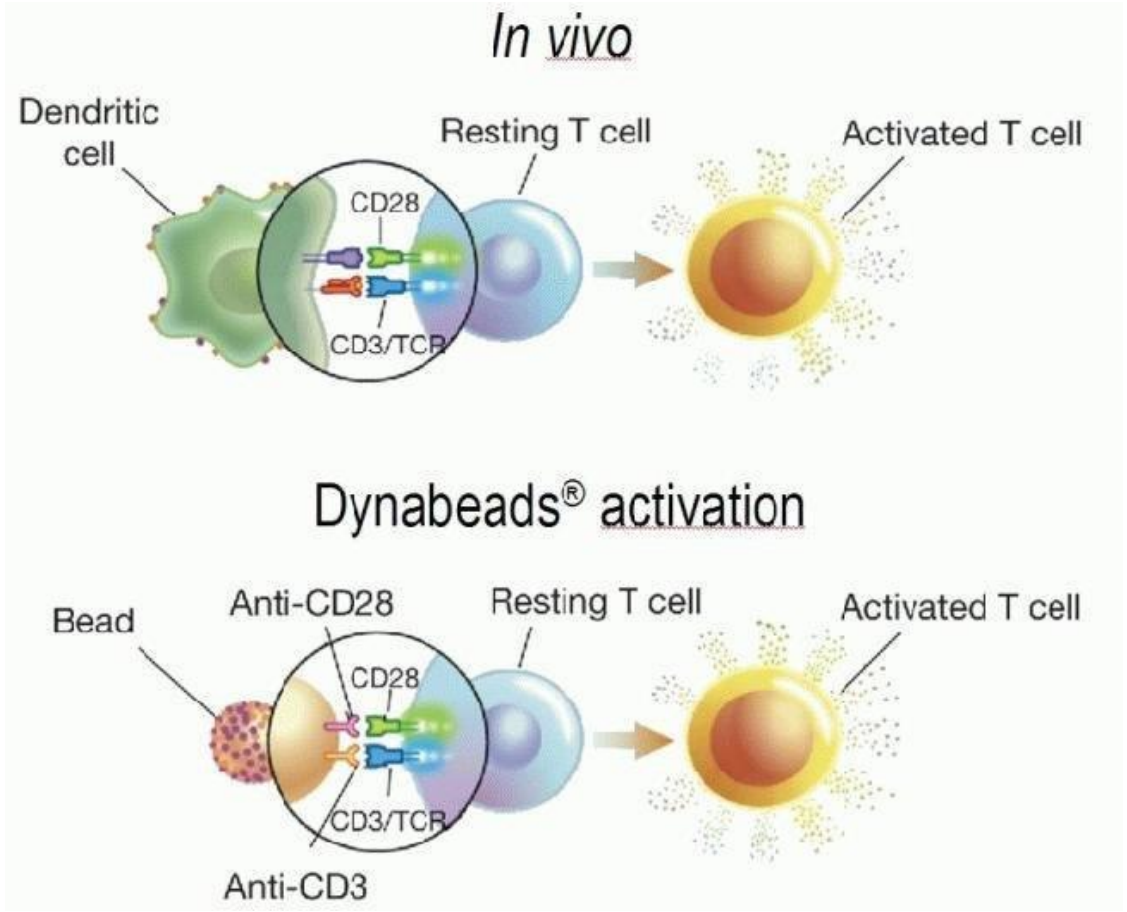
Polyfunctionality

- Cells are functional
- 31% produce all 3 cytokines


Day 0
Day 7
Day 14
CD62L
CD45RA
22.2
21.7
25.2
30.9
82.9
12.2
4.14
0.72
8.61
39.8
37.7
13.9

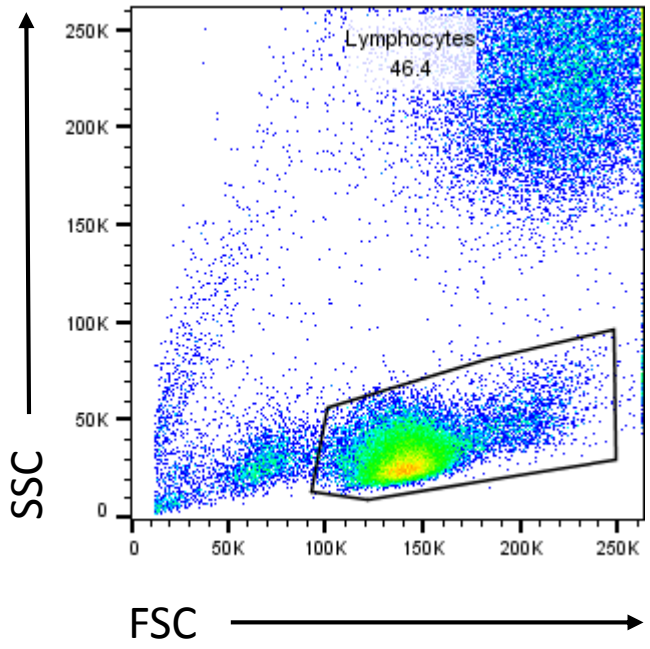

How Can We Generate More Cells?
SHIBUTAN
DHC
TSUTAYA
STARBUCKS COFFEE
Euphoria

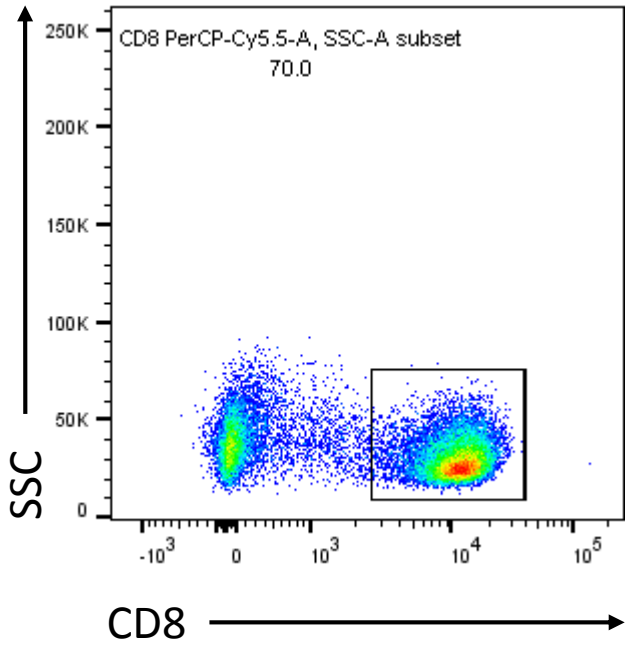
CD3/CD28 aAPC

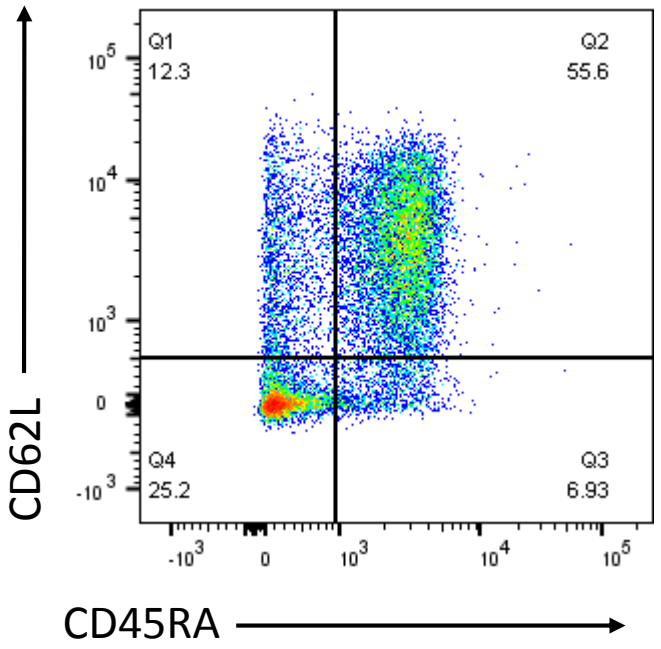


https://www.thermofisher.com/order/catalog/product/11161D

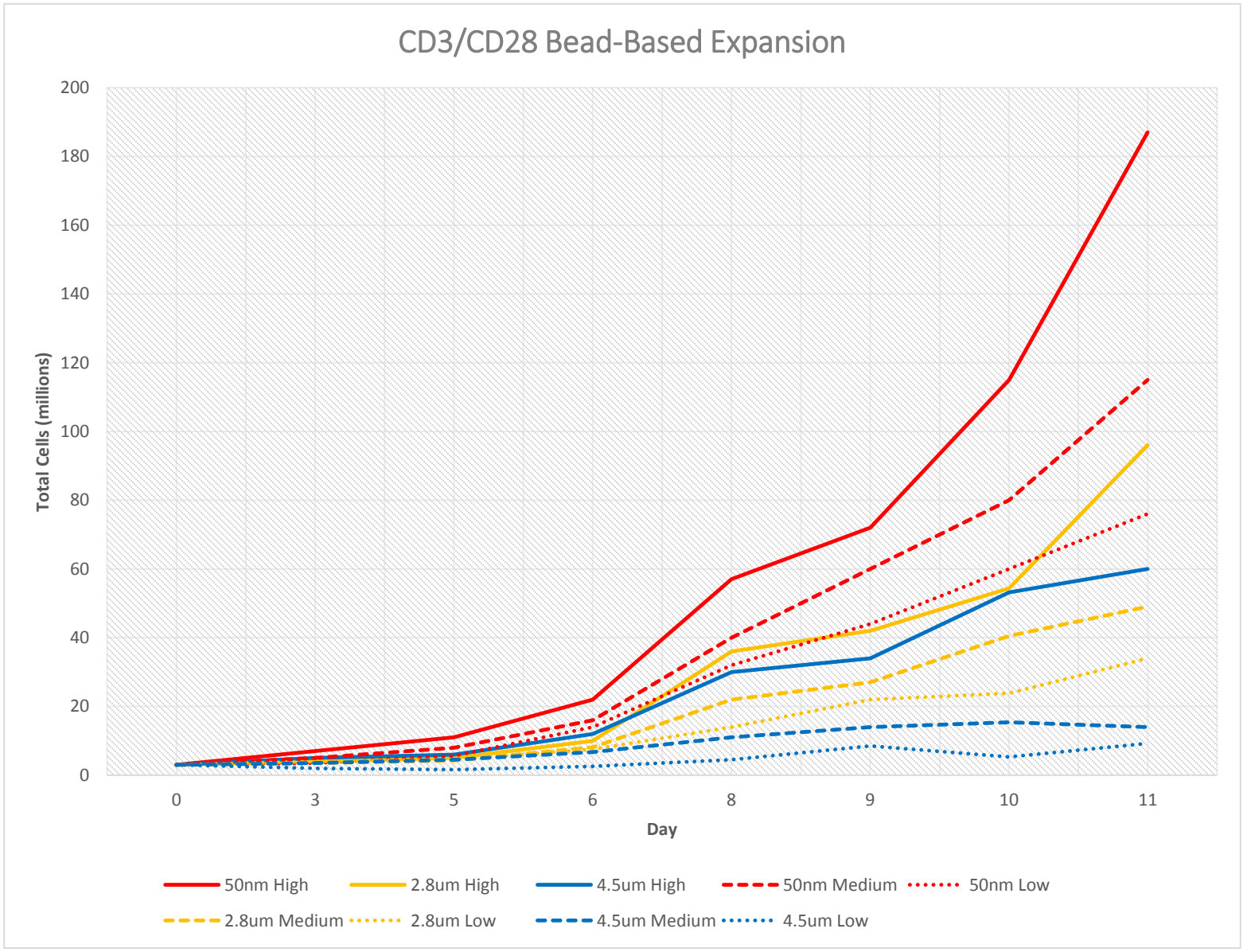
Day 0 Memory Phenotype







Nanoscale Bead Performance



Day 10

50nm

2.8µm

4.5µm

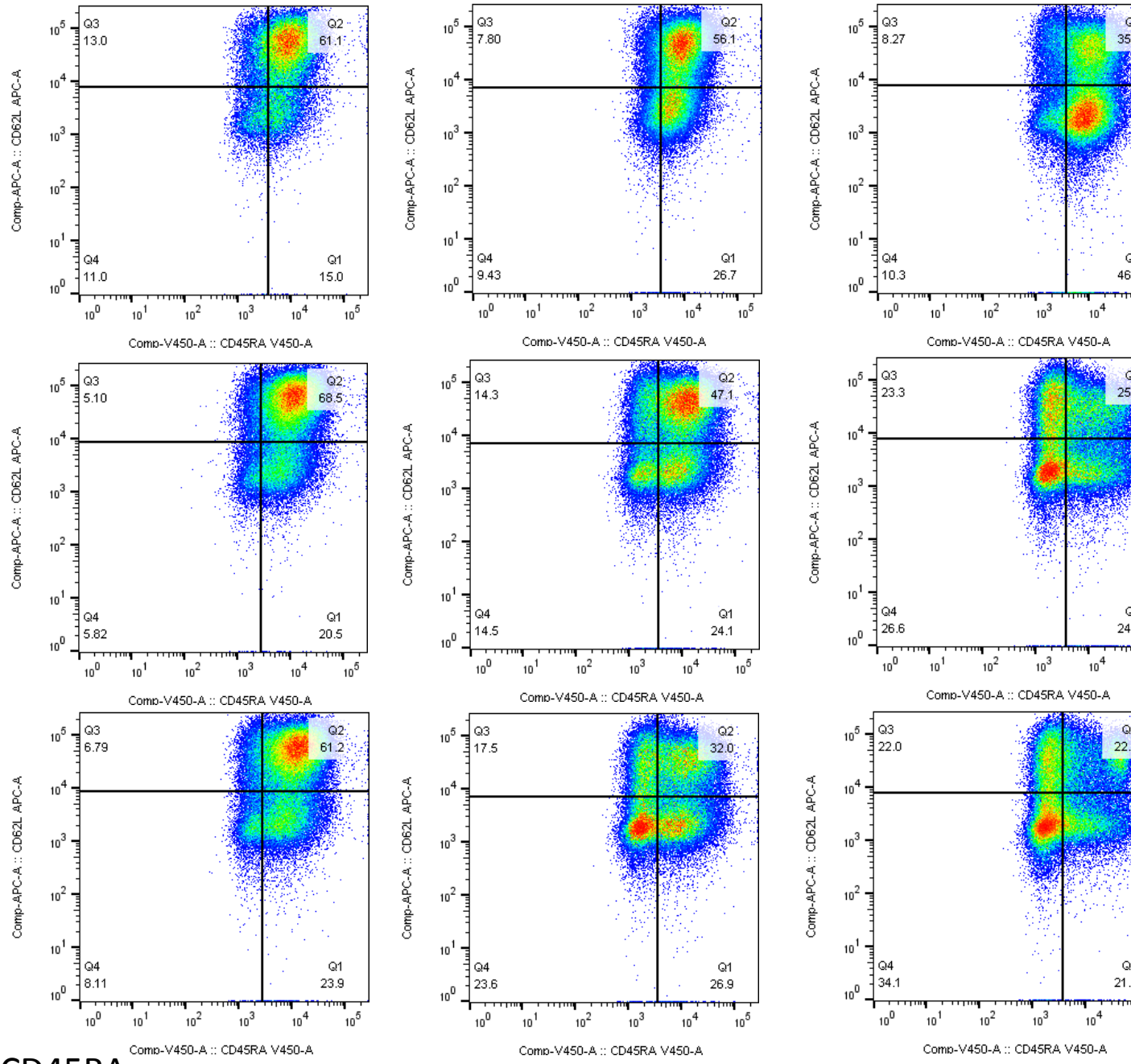
High
30uL/1E6

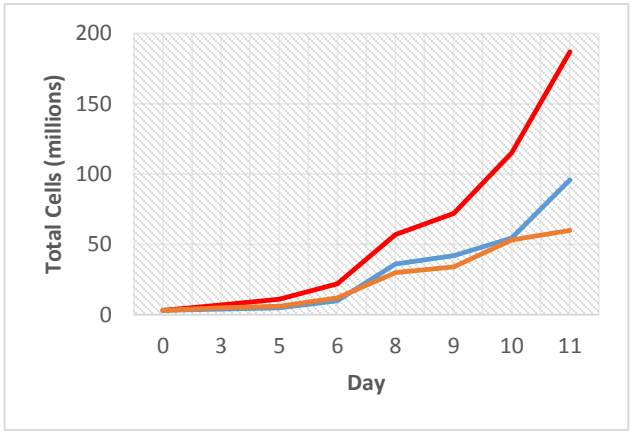
Medium
15uL/1E6

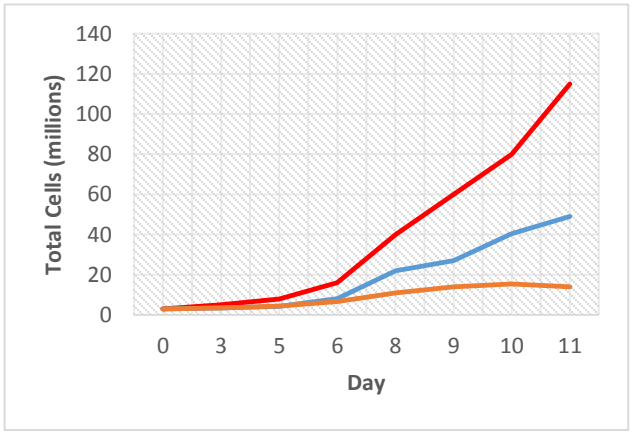
Low
5uL/1E6

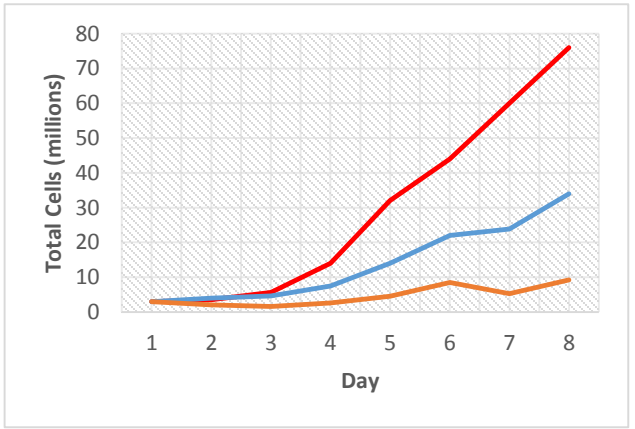
CD62L

CD45RA









Conclusions

- Bead-bound tetramer enriches cells of interest
- Bead-bound tetramer is stimulatory and mediates robust expansion
- Expanded cells are polyfunctional
- Nanoscale CD3/CD28 beads are effective for CD8+ T cell expansion

Future Directions

- Vary signal 2
- Optimize cytokines
- Go for rare antigens!
- Expand CD3+ and PBMC

Acknowledgments

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