

WuXi *In Vitro* Immunology Assay Platform



WuXi AppTec Research Service Division, Oncology & Immunology Unit



2020.08

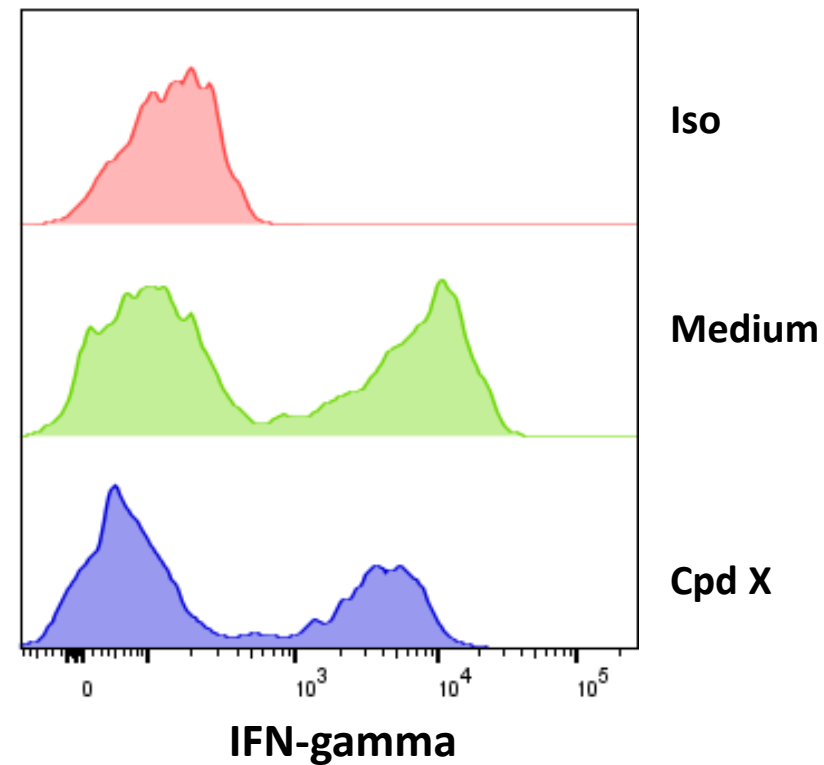
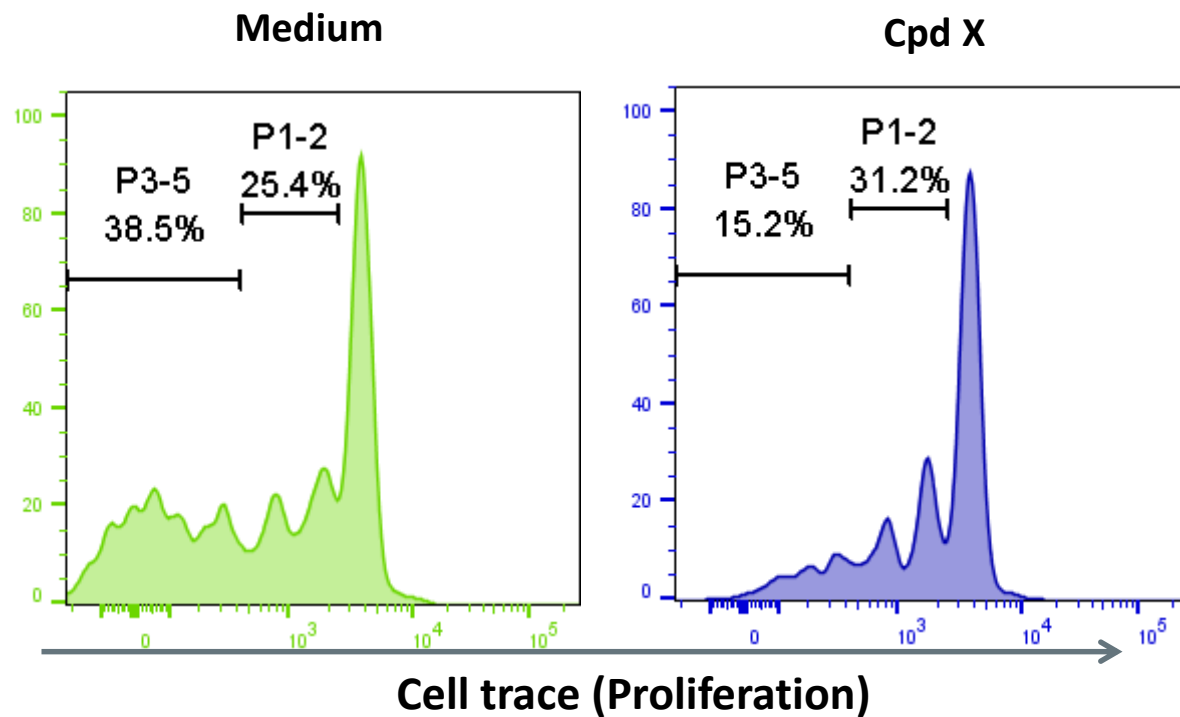
OncoWuXi Newsletter

Service list of *In-vitro* and *ex-vivo* immunology assays

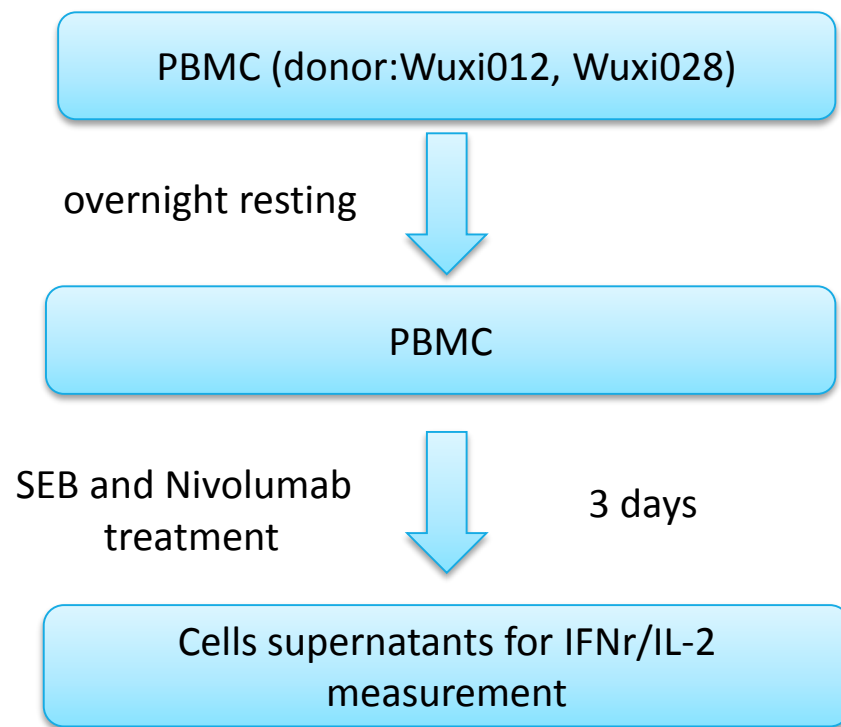
Assay			WuXi capability (Human/Mouse)
Cell Activation		T Cell Agonism (Antibody/SEB/PHA/CMV Recall)	Yes (H/M)
		B Cell Activation	Yes (H/M)
		NK Cell Activation/Expansion	Yes (H/M)
Cell Differentiation	T Cell Polarization	Treg	Yes (H/M)
		TH1	Yes (H/M)
		TH2	Yes (H/M)
		TH17	Yes (H/M)
	Macrophage Differentiation	M1/M2 Macrophage	Yes (H/M)
		MDSC	Yes (H)
		DC/Tumor activate DC	Yes (H/M)
Cytotoxicity Assay	T Cell	OT-1 system (M)/CAR T cell system (H)	Yes (H/M)
	NK Cell	NK cell cytotoxicity	Yes (H)
		ADCC/CDC/ADCP	Yes (H)
Mixed Lymphocyte Reaction		DC co-culture with T	Yes (H/M)
Immune Cell Suppressive Assay		MDSC suppressive assay	Yes (H)
		Treg suppressive assay	Yes (H)
		Tumor cell suppressive assay (co-culture with immune cells)	Yes (H)
		Sting pathway activation	Yes (H)
Other assay		NLRP3 inflammasome activation assay	Yes (H)
		Antigen specific T cell stimulation in vivo (OT1 system)	Yes (M)

T Cell Activation by Antibody

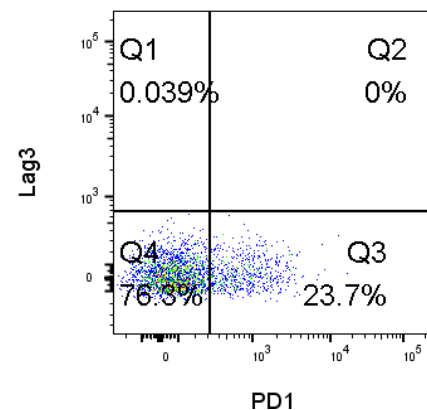
Anti-CD3/CD28 Stimulation



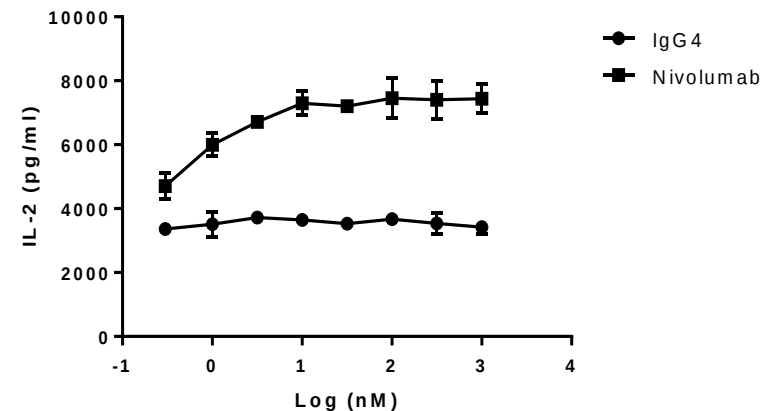
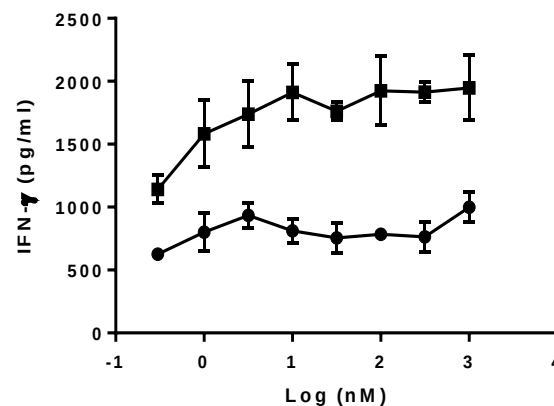
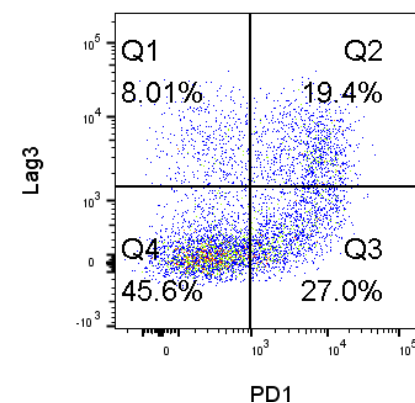
T Cell Activation by SEB



Before stimulation



After stimulation



T Cell Activation by PHA

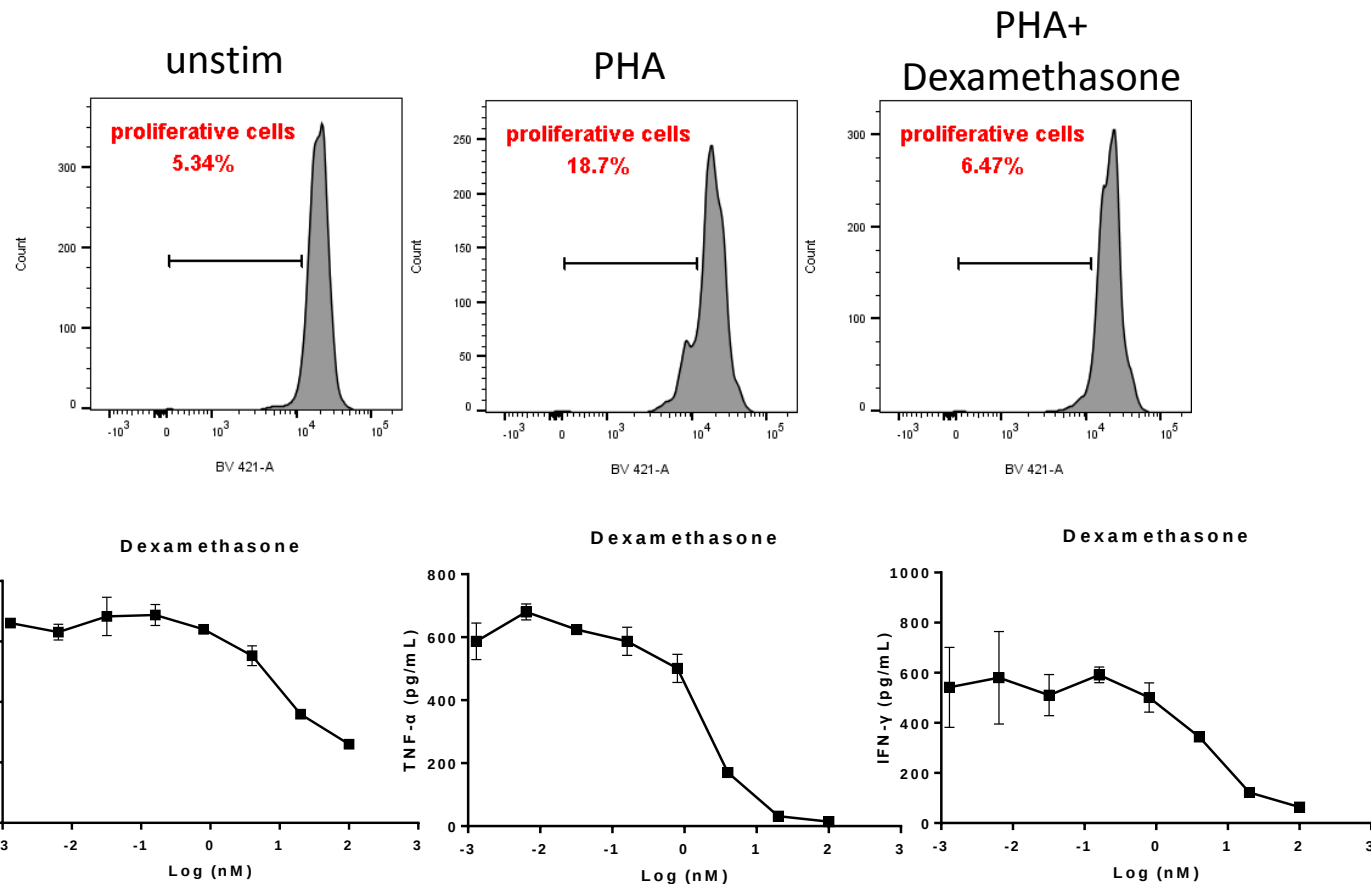
Day0: Recover PBMC, stain with cell-trace violet



PHA to stimulate PBMC in the presence of tested articles



Cells for proliferation and supernatant for IFN- γ and TNF- α

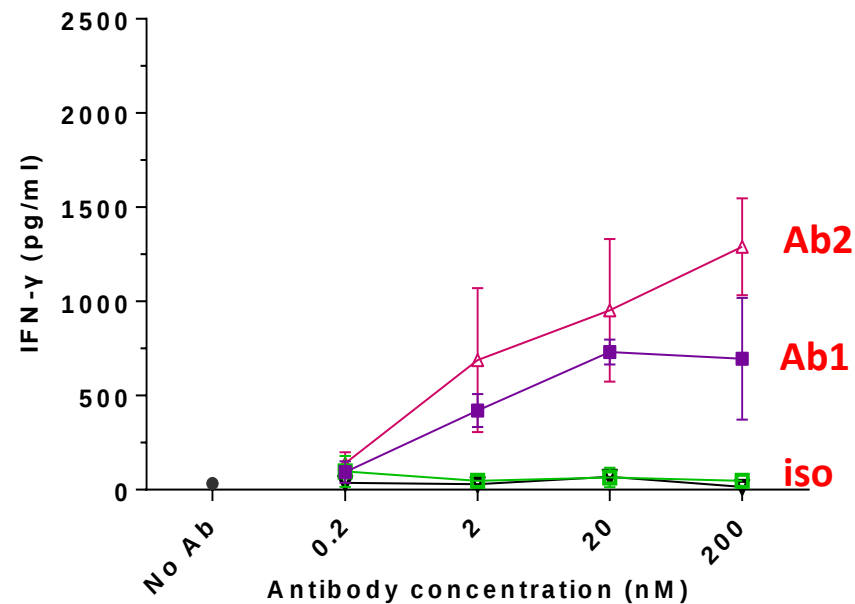
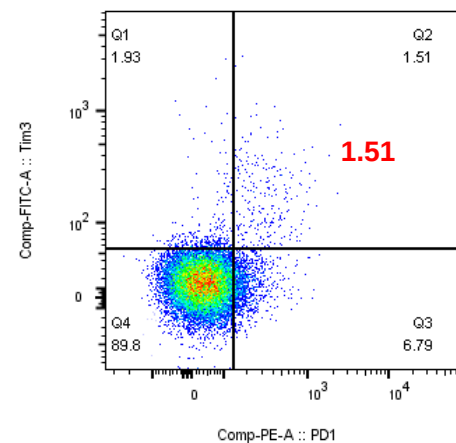
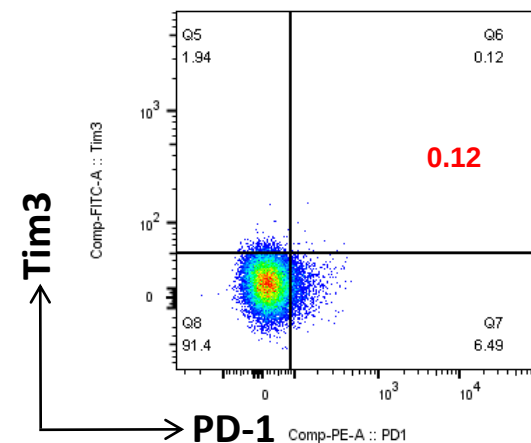
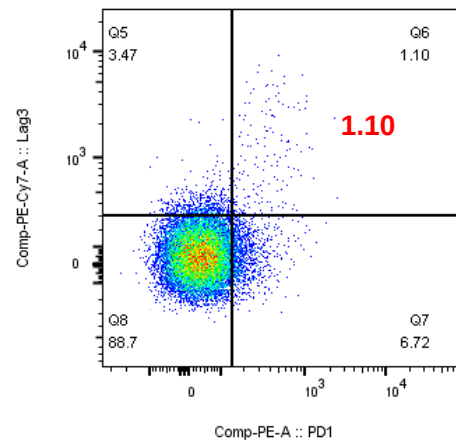
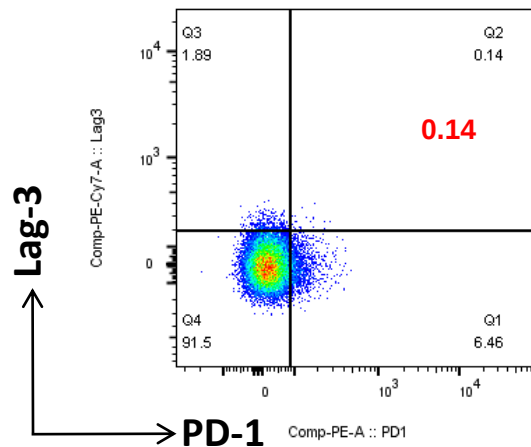


CMV Recall Assay

CD4+ T

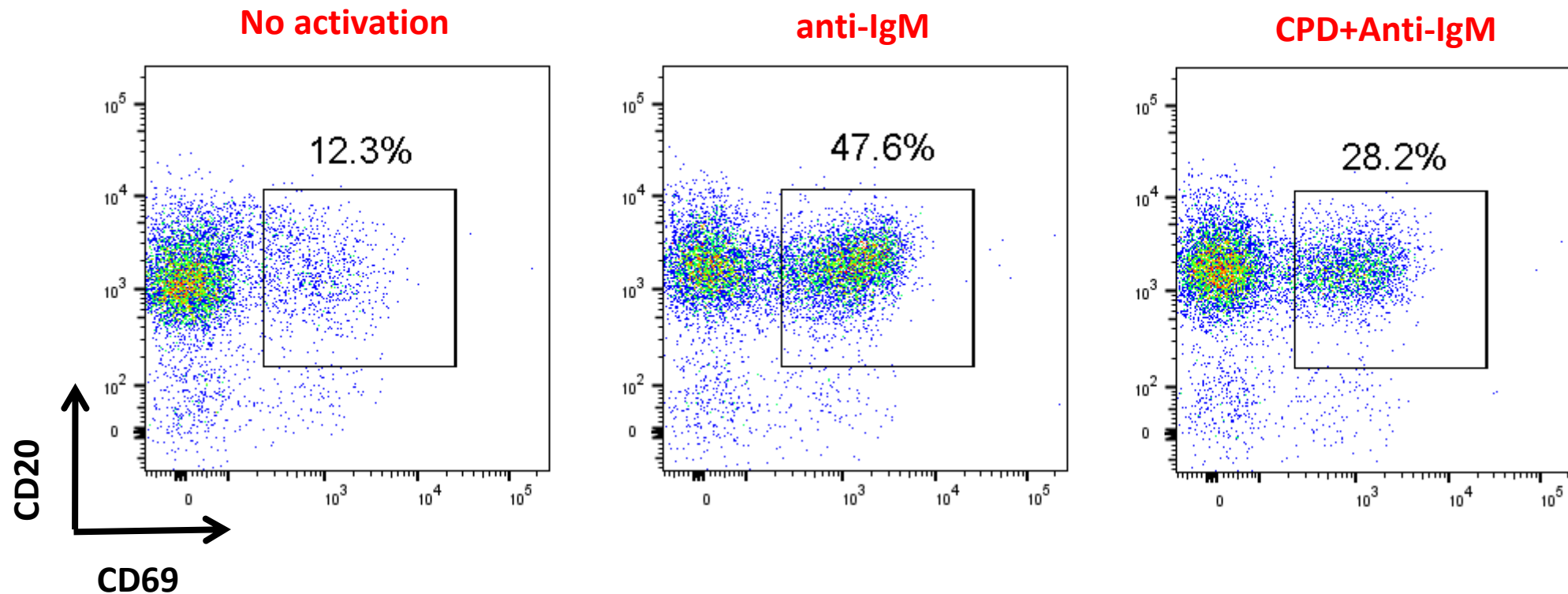
Before stimulation

After stimulation

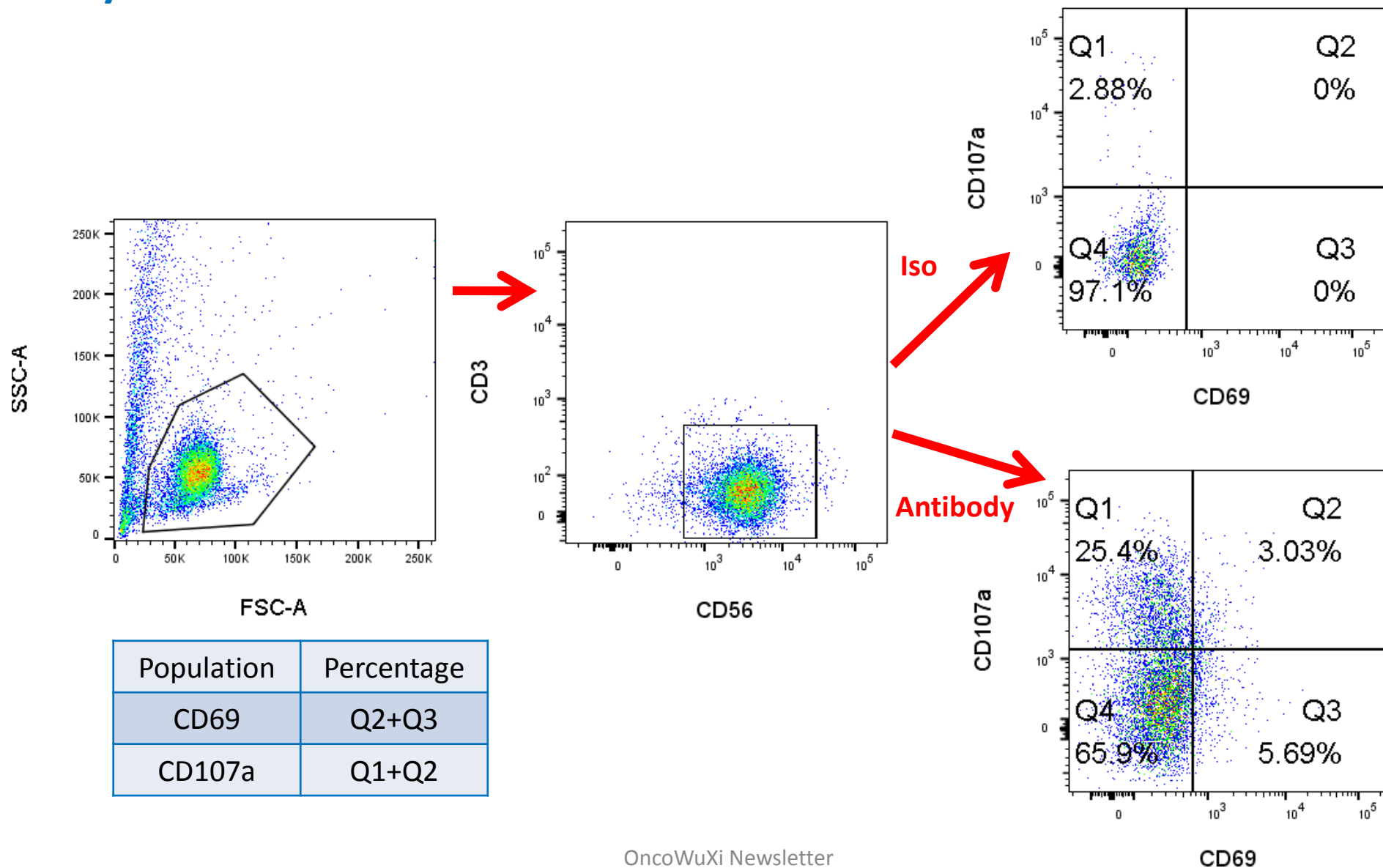


B Cell Activation

Anti-IgM Stimulation

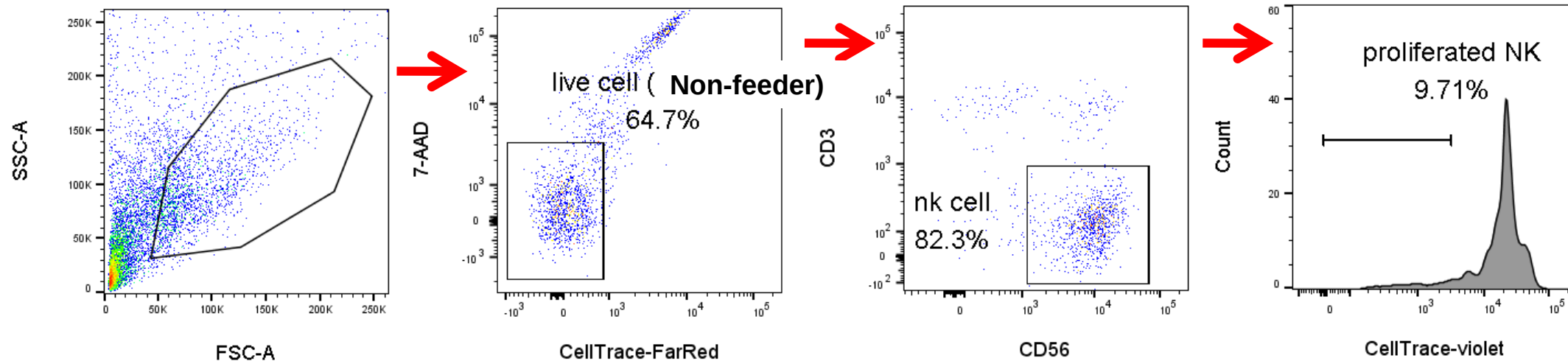


Primary NK Activation

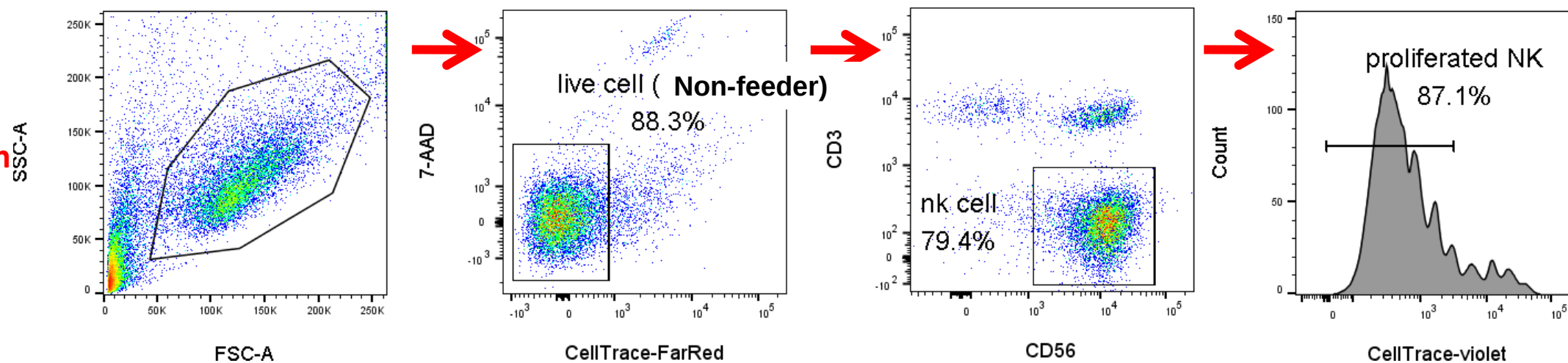


Primary NK Expansion

No
stimulus



stimulation



T helper Cell Polarization

Treg differentiation

Day 0

Isolation of naïve CD4⁺ T cells from PBMC

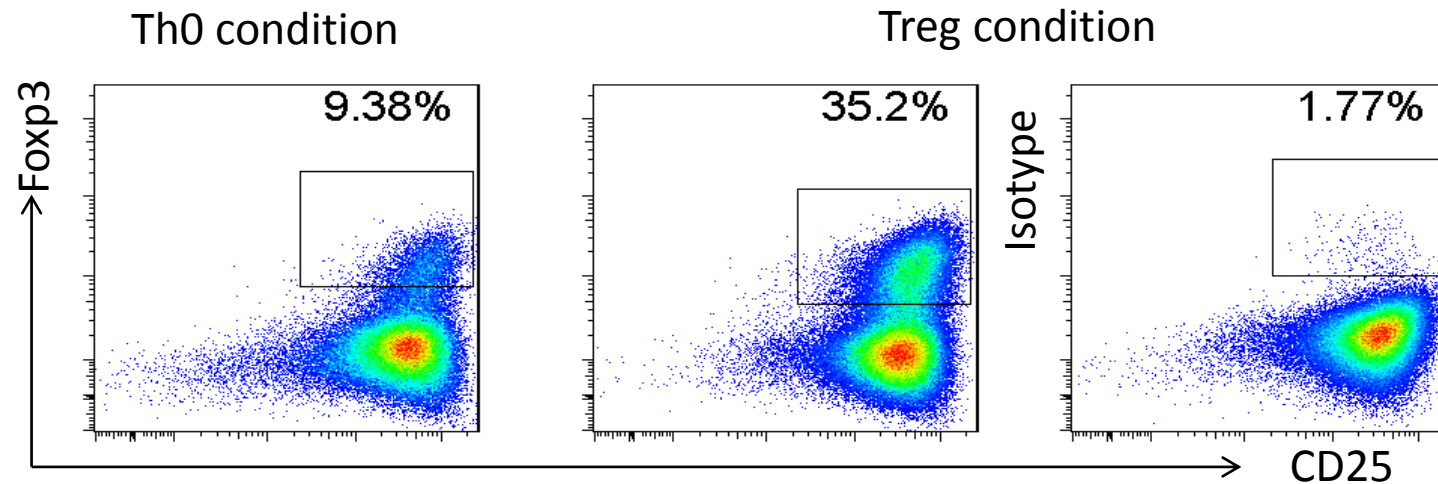


Stimulation with anti-CD3/CD28 in the presence of
Treg differentiation cocktail



Day 5

FACS (LIVE-DEAD/CD4/CD25/FoxP3)



T helper Cell Polarization

Th1 differentiation

Day 0

Isolation of naïve CD4⁺ T cells from PBMC

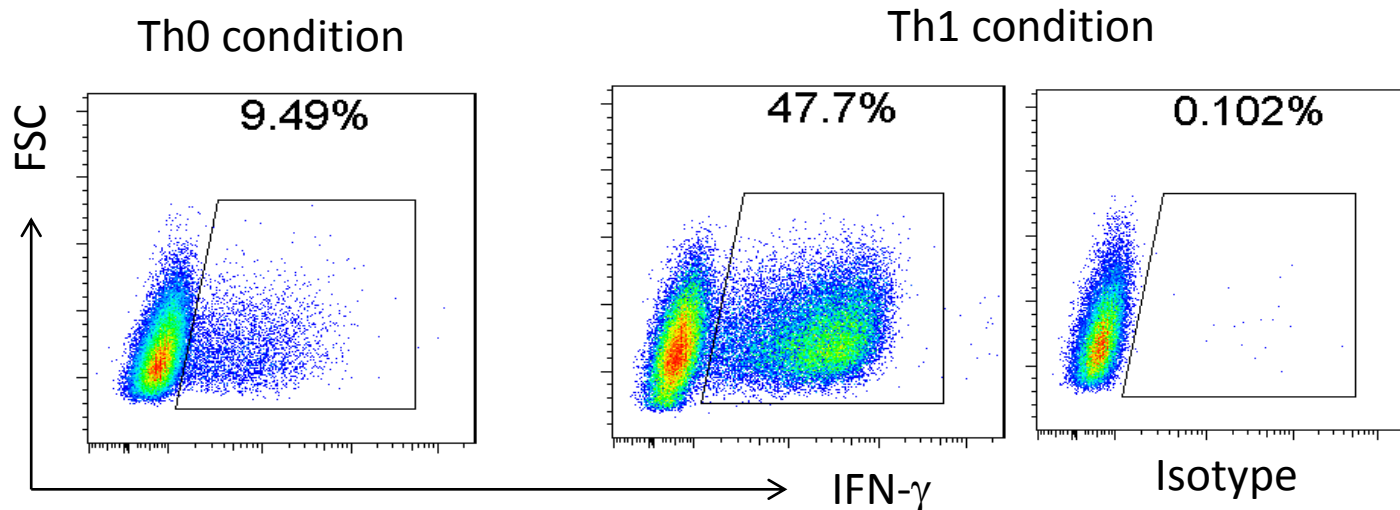


Stimulation with anti-CD3/CD28 Dynabeads in the presence of Th1 differentiation cocktail



Day 5

FACS (LIVE-DEAD/CD4/IFN- γ)



T helper Cell Polarization

Th2 differentiation

Day 0

Isolation of naïve CD4⁺ T cells from PBMC

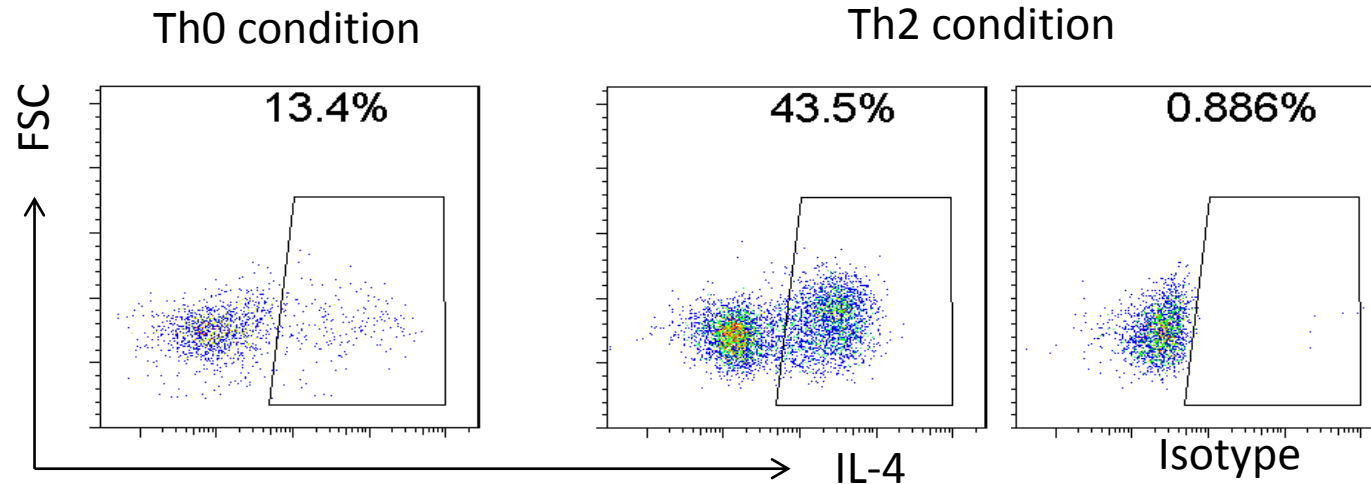


Stimulation with anti-CD3/CD28 in the presence of
Th2 differentiation cocktail



Day 13

FACS (LIVE-DEAD/CD4/IL-4)



T helper Cell Polarization

Th17 differentiation

Day 0

Isolation of naïve CD4⁺ T cells from PBMC

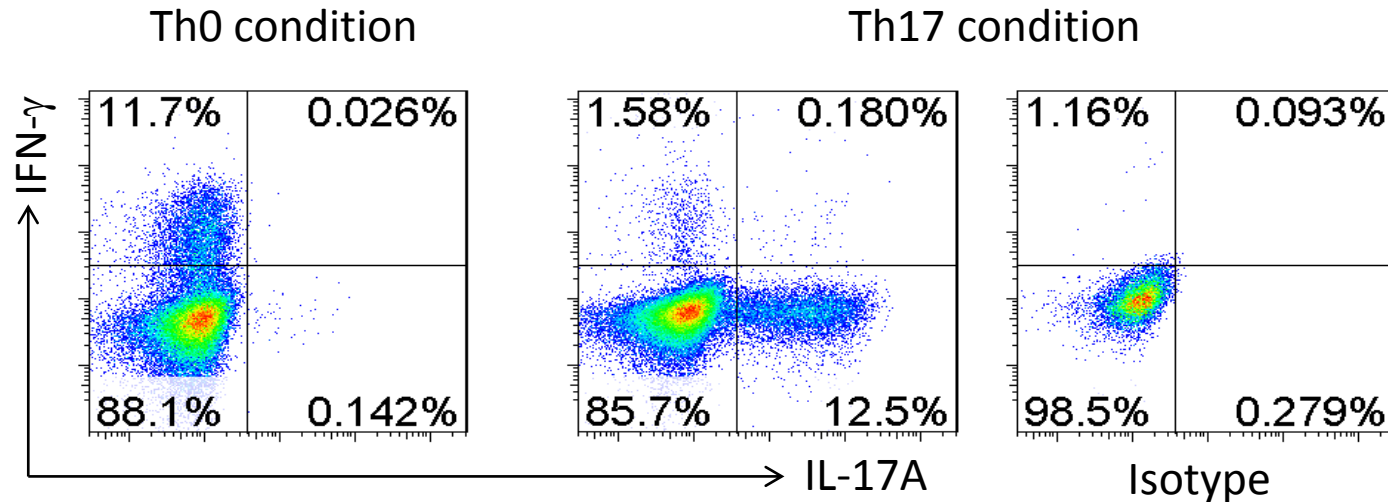


Stimulation with anti-CD3/CD28 in the presence of
Th17 differentiation cocktail



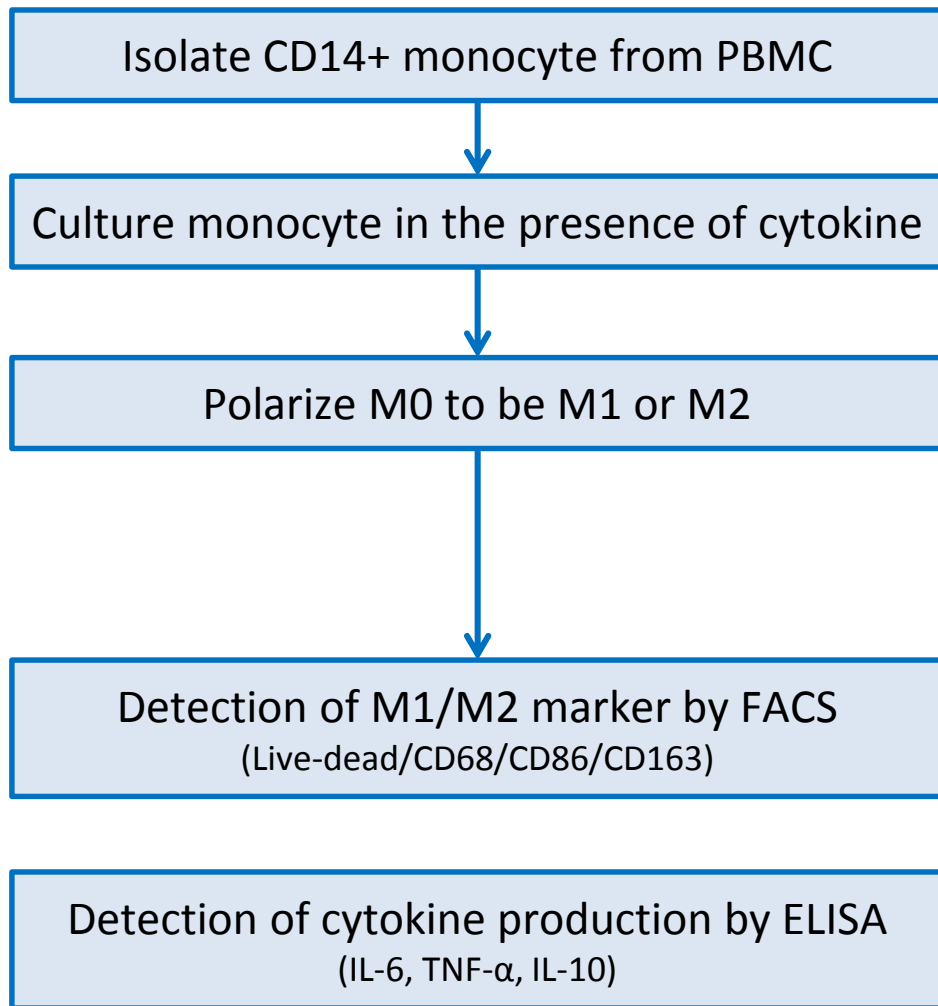
Day 11

FACS (LIVE-DEAD/CD4/IL-17A)

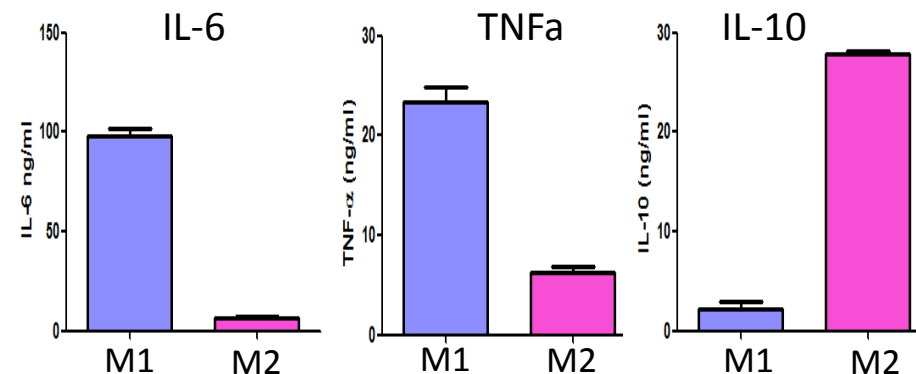
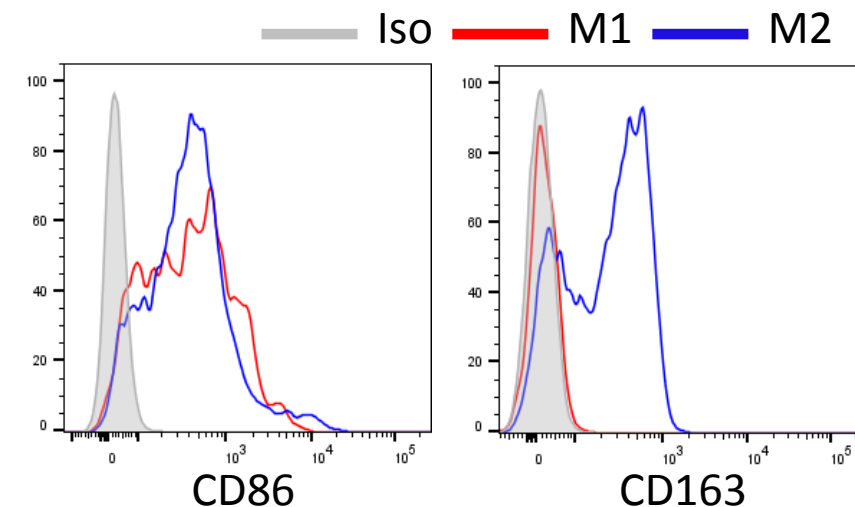


Macrophage Differentiation

Human M1/M2 macrophage differentiation

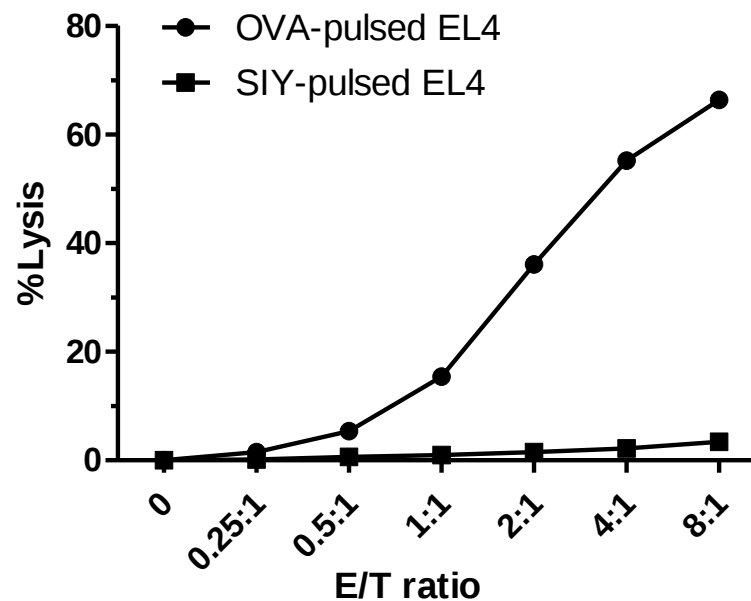
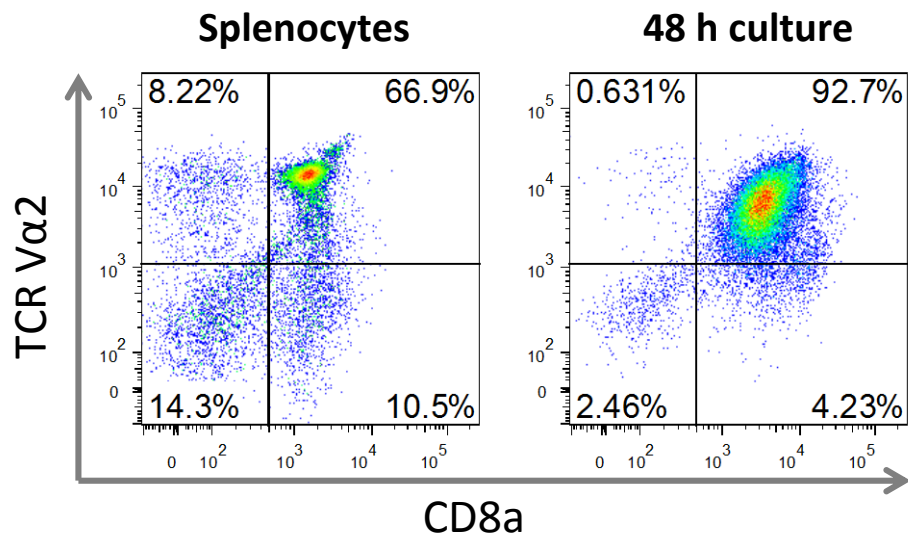


FACS analysis of M1/M2 macrophage:



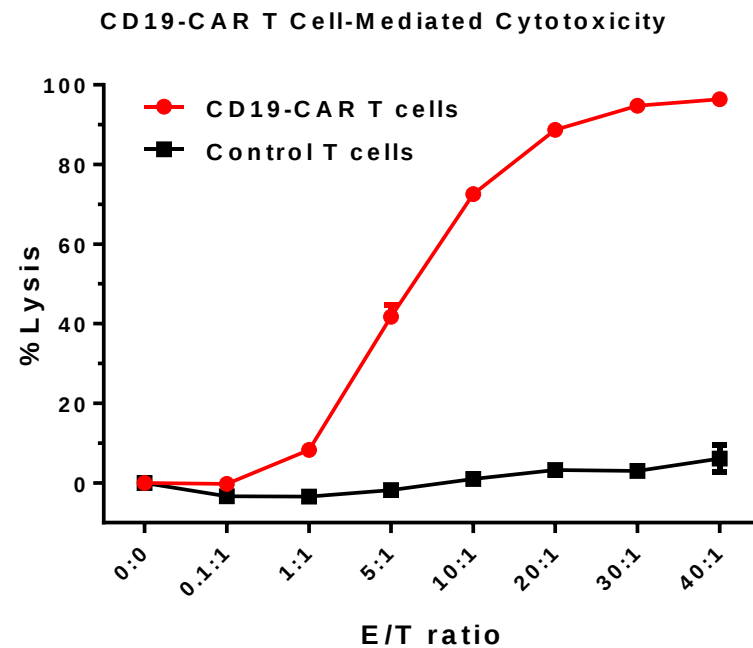
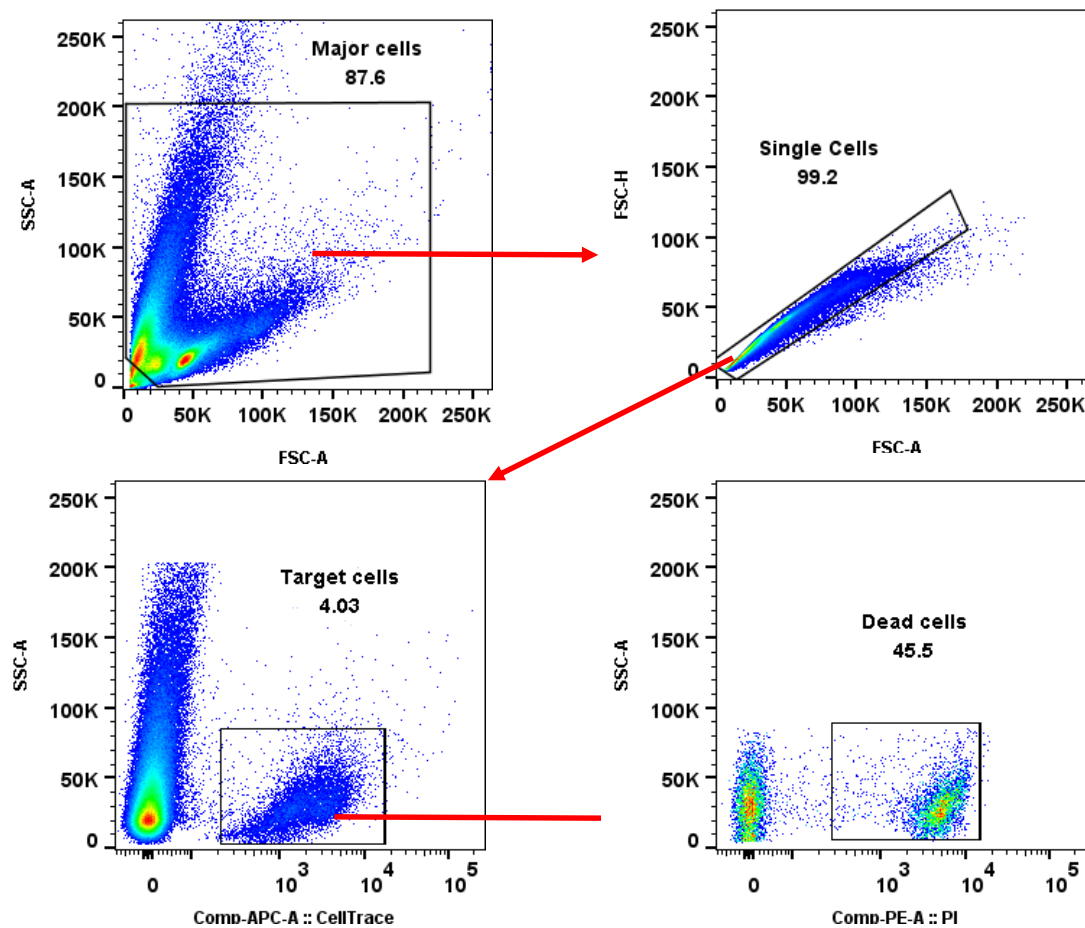
CTL Cytotoxicity Assay (OT-I System)

- **Effector:** Antigen-specific OT-I splenocyte culture
- **Target:** CellTrace Far-Red labeled EL4 cells



CD19-CAR T Cell-Mediated Cytotoxicity Assay

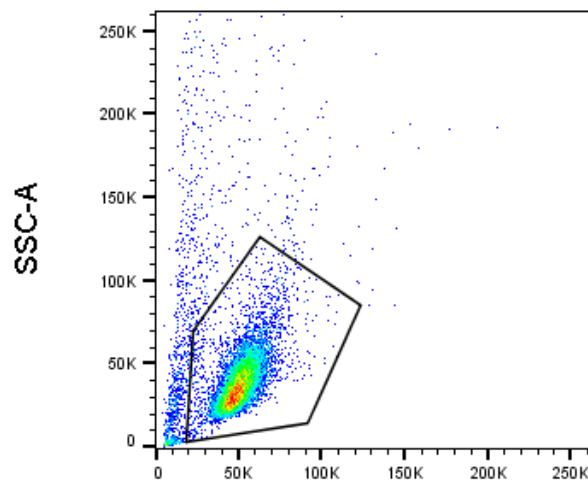
- **Effector:** CD19-CAR T cells
- **Target:** CellTrace Far-Red labeled Raji cell



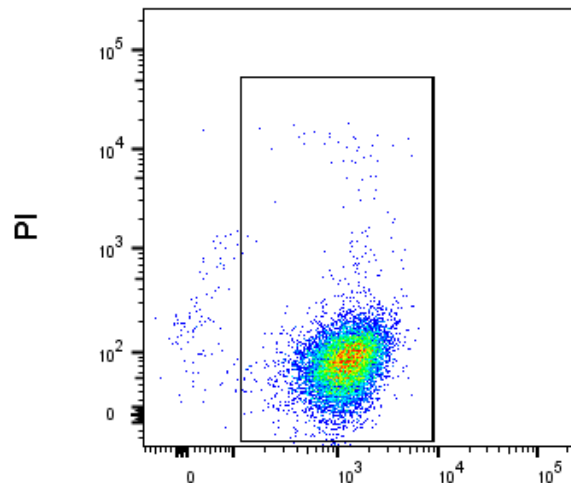
NK92MI Cyto-K562

K562 cells

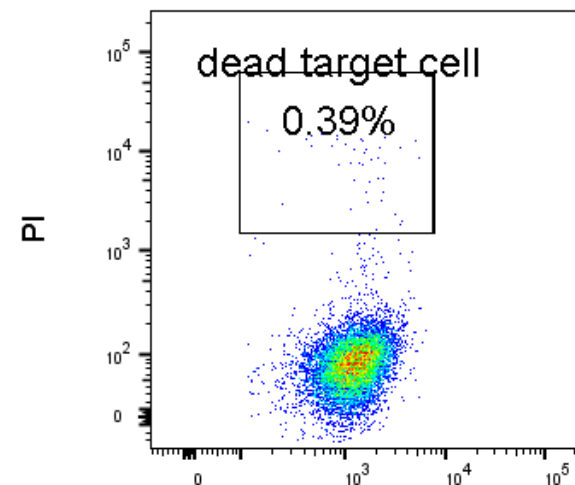
K562 only



FSC-A

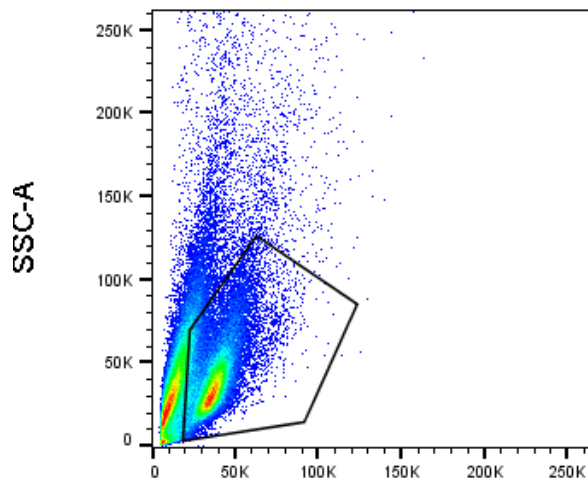


CellTrace

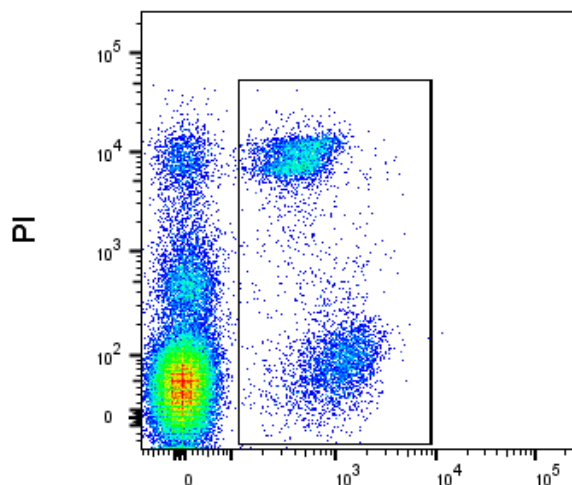


CellTrace

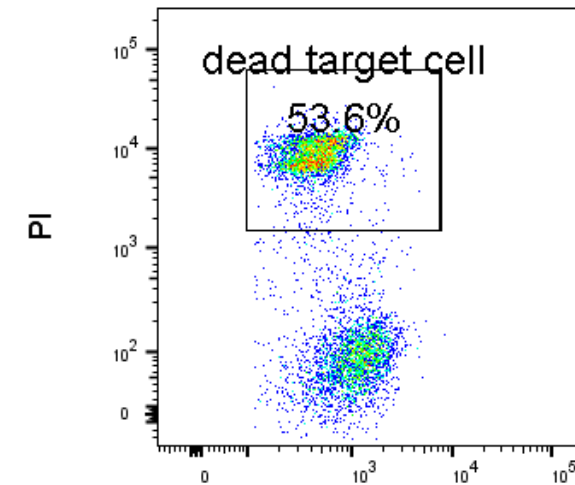
NK92MI+K562



FSC-A



CellTrace

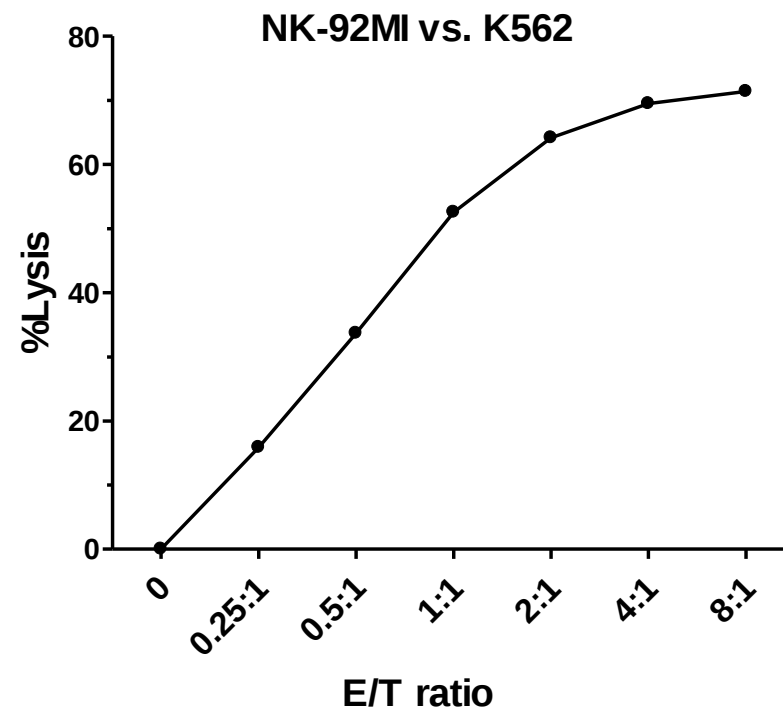
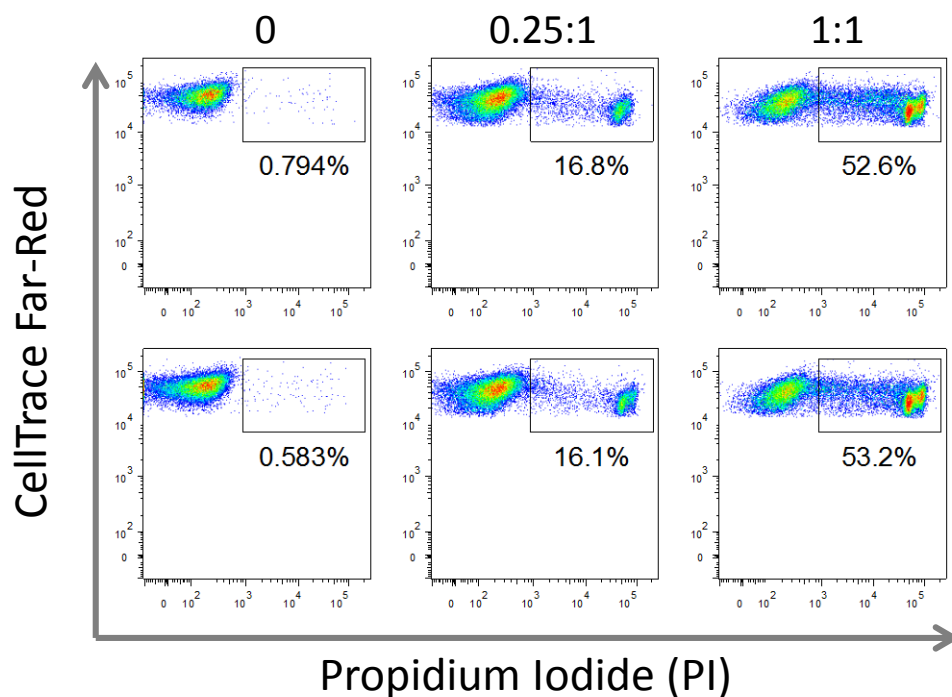


CellTrace

NK Cytotoxicity Assay (NK-92MI Cell Line)

- **Effector:** NK-92MI cells
- **Target:** K562 cells labeled with 100 nM CellTrace Far-Red

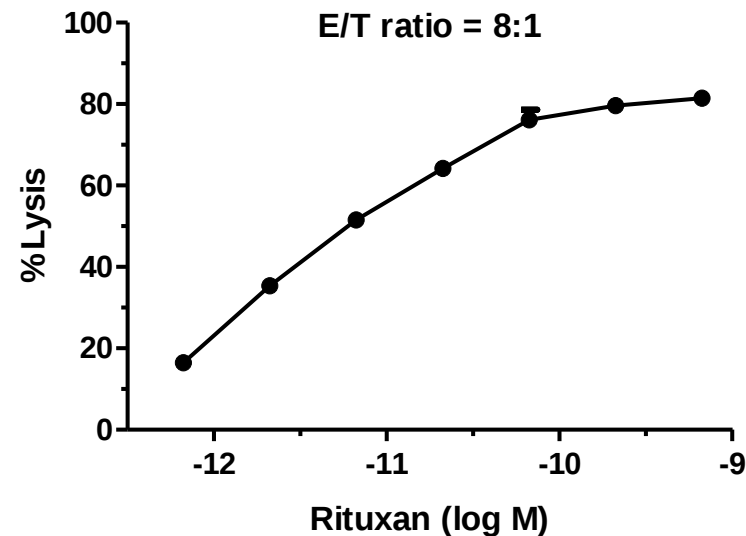
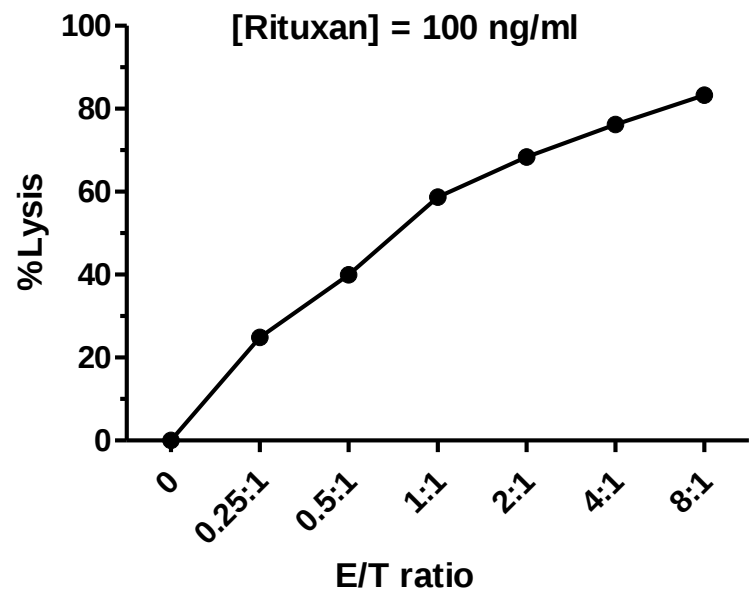
Duplicate wells of E/T ratios:



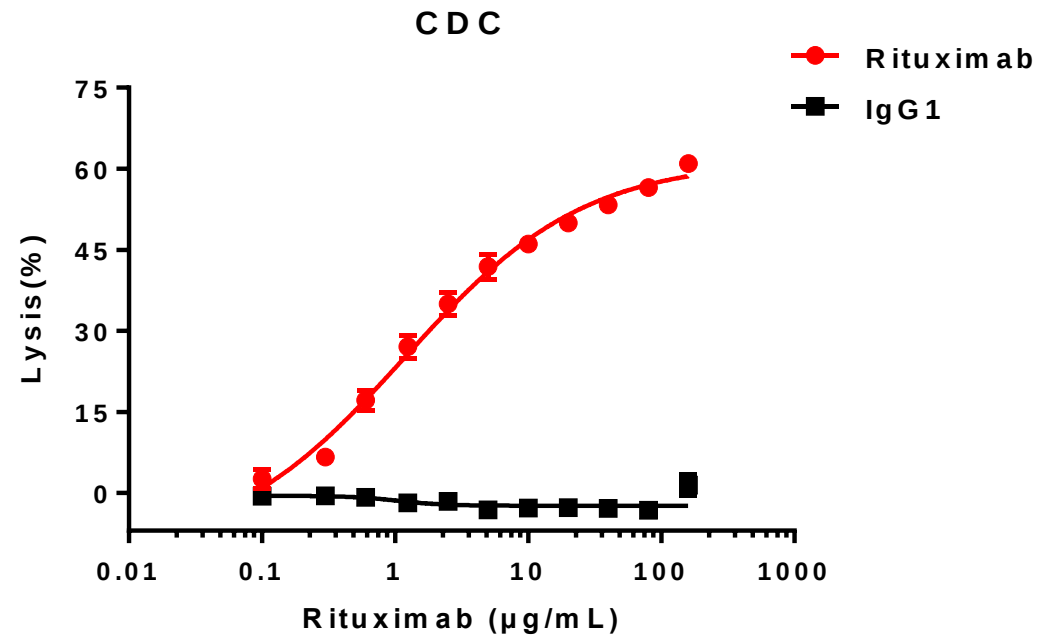
ADCC Assay (Purified Primary NK cells)

Antibody-Dependent Cell-Mediated Cytotoxicity

- **Effector:** NK cells isolated from PBMC of healthy donor
- **Target:** CD20⁺ Raji cell line, labeled with 50 nM CellTrace Far-Red

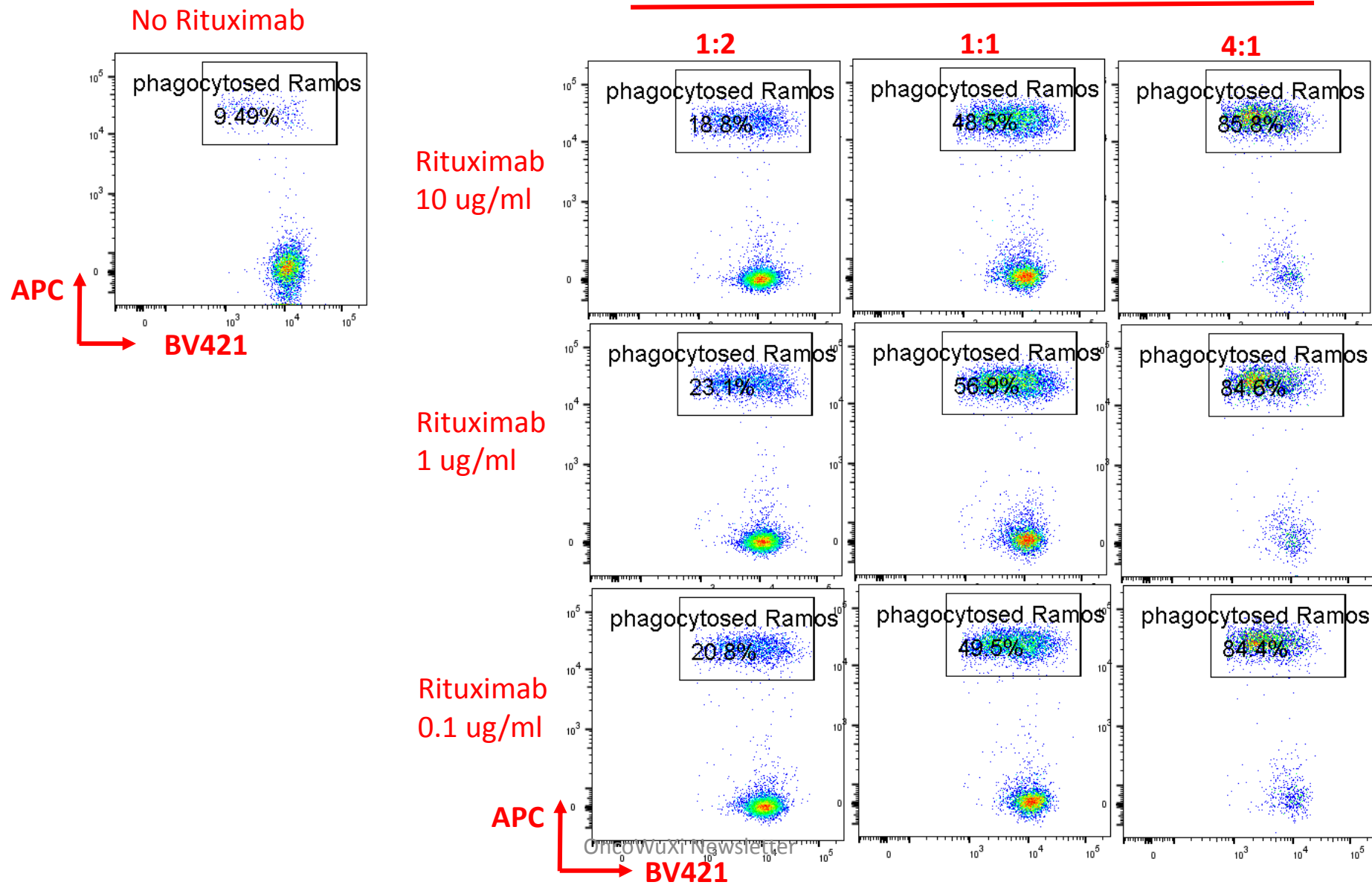


- **Effector:** Complement in human serum
- **Target:** Daudi cells



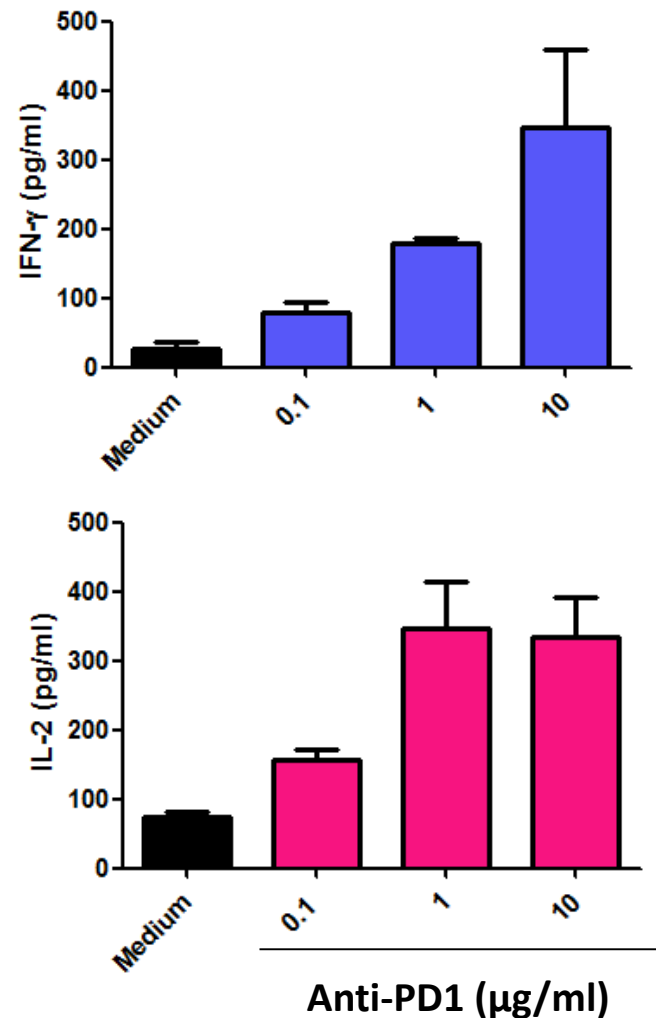
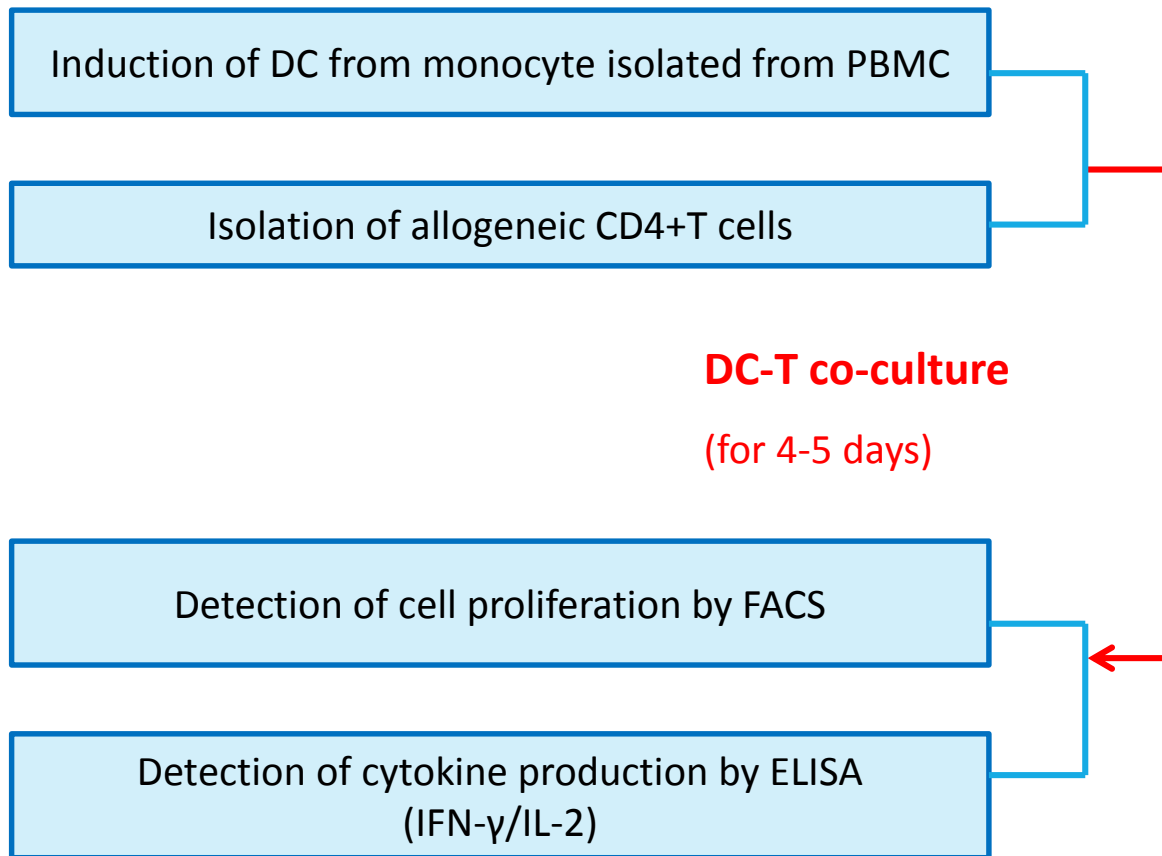
ADCP Assay-M-CSF induced macrophage

Macrophage:Ramos



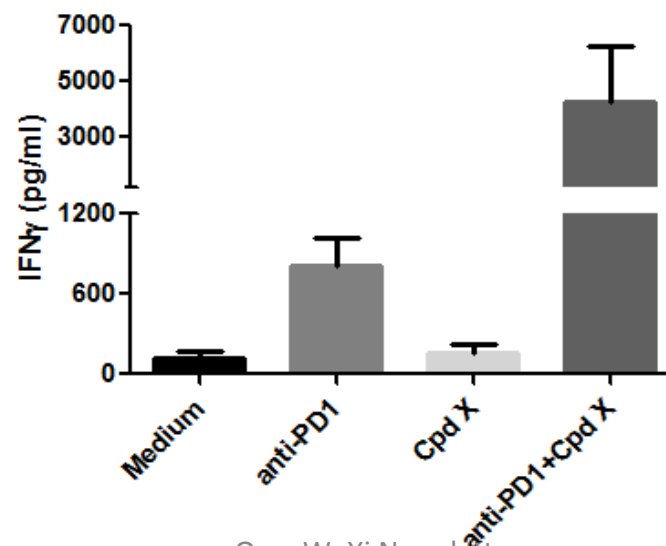
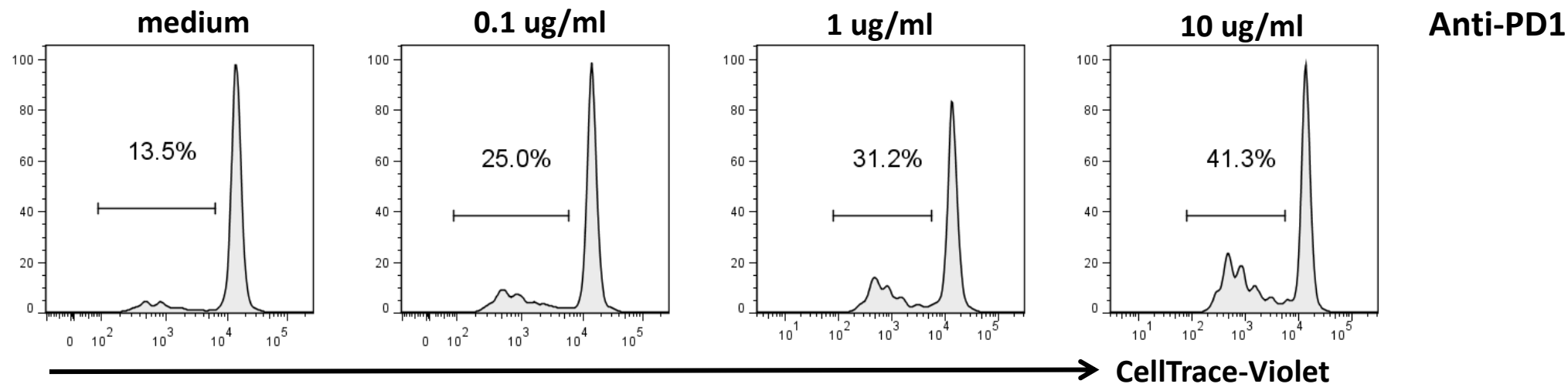
Mixed Lymphocyte Reaction

DC co-cultured with T cell



Mixed Lymphocyte Reaction

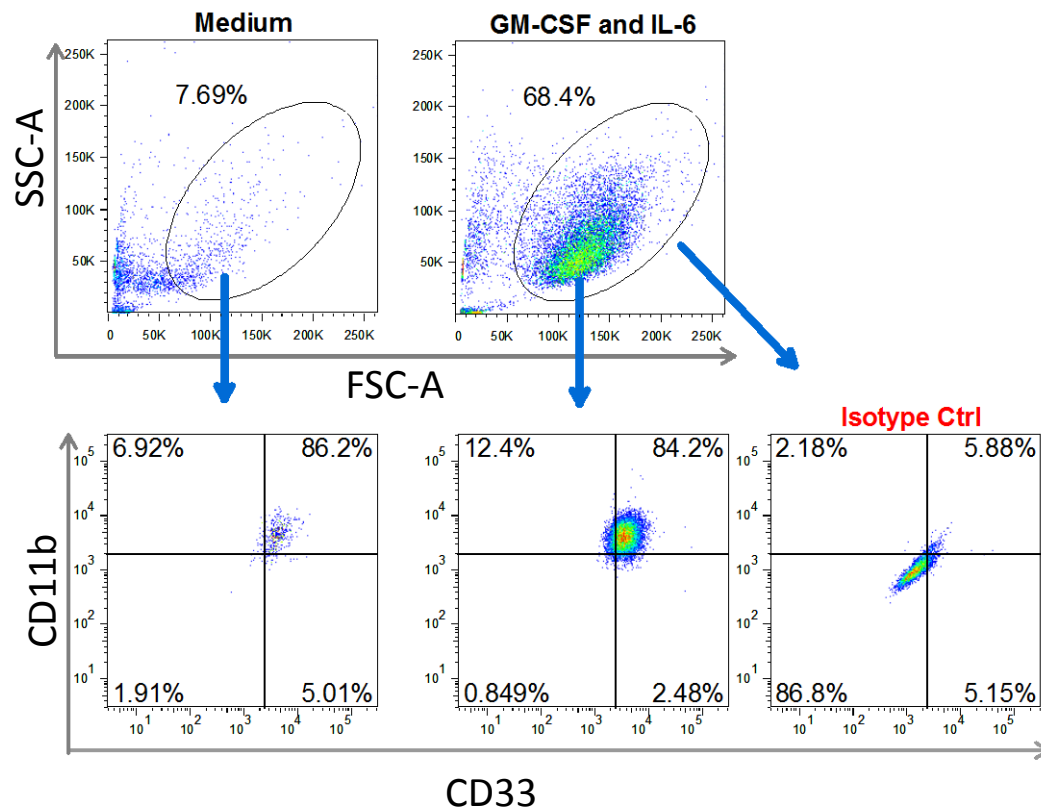
DC co-cultured with T cell



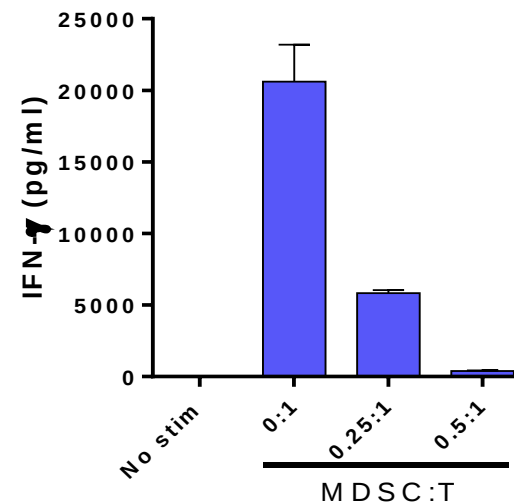
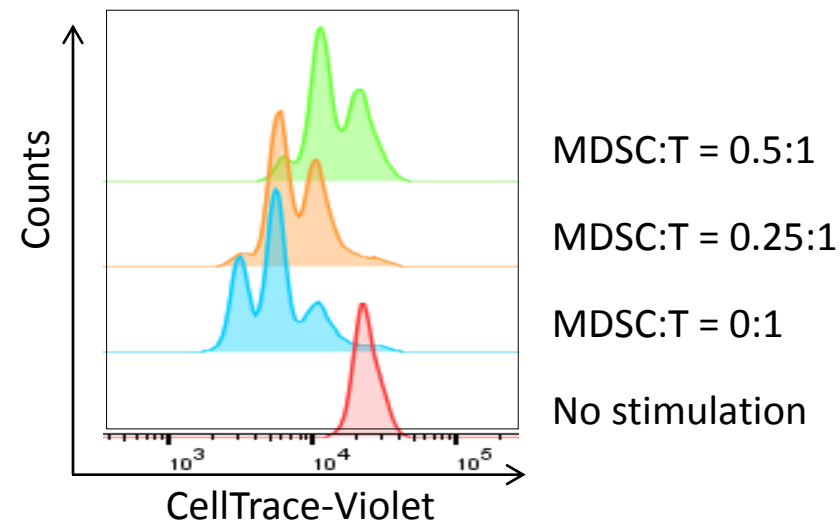
MDSC Suppressive Assay

MDSC Induction and Co-culture with T Cell

Induction of MDSC from PBMC

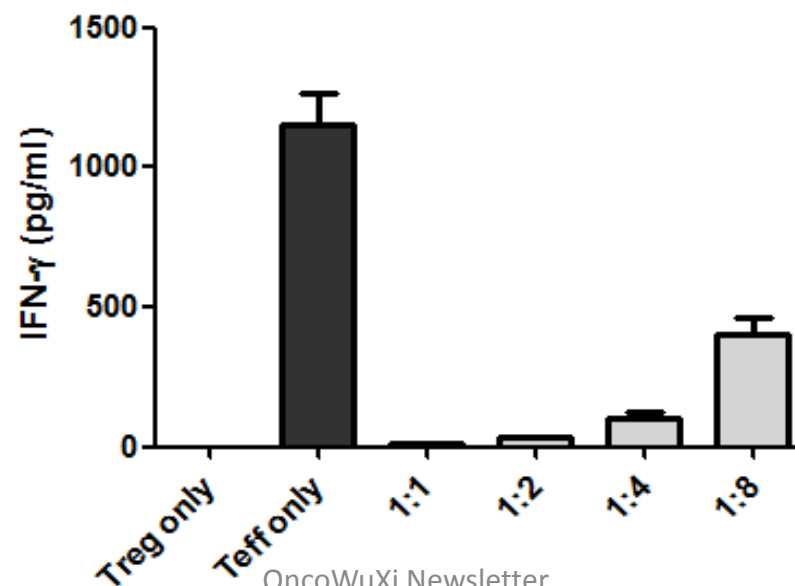
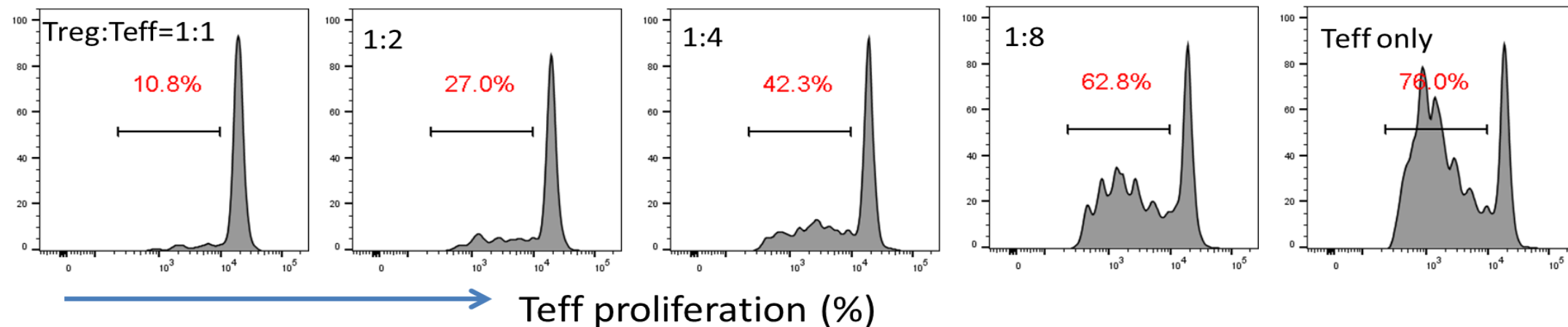


MDSC suppress T cell proliferation and cytokine production in co-culture



Treg Suppressive Assay

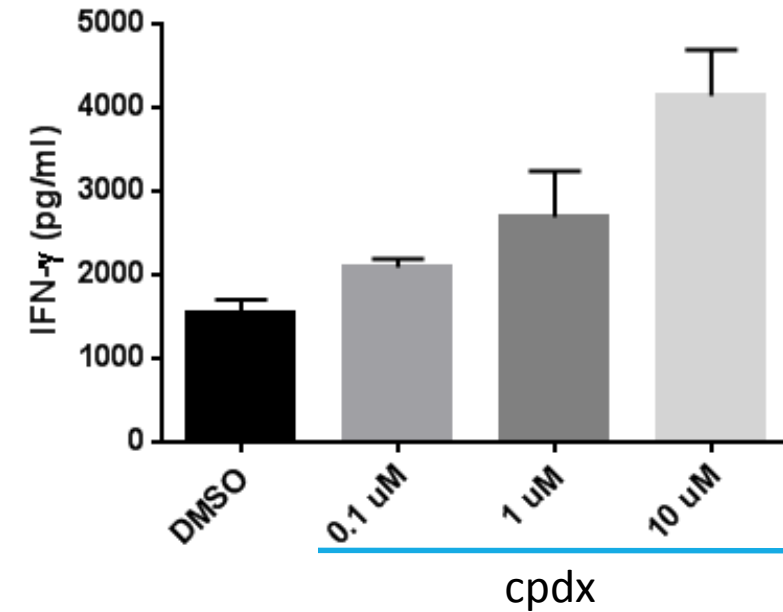
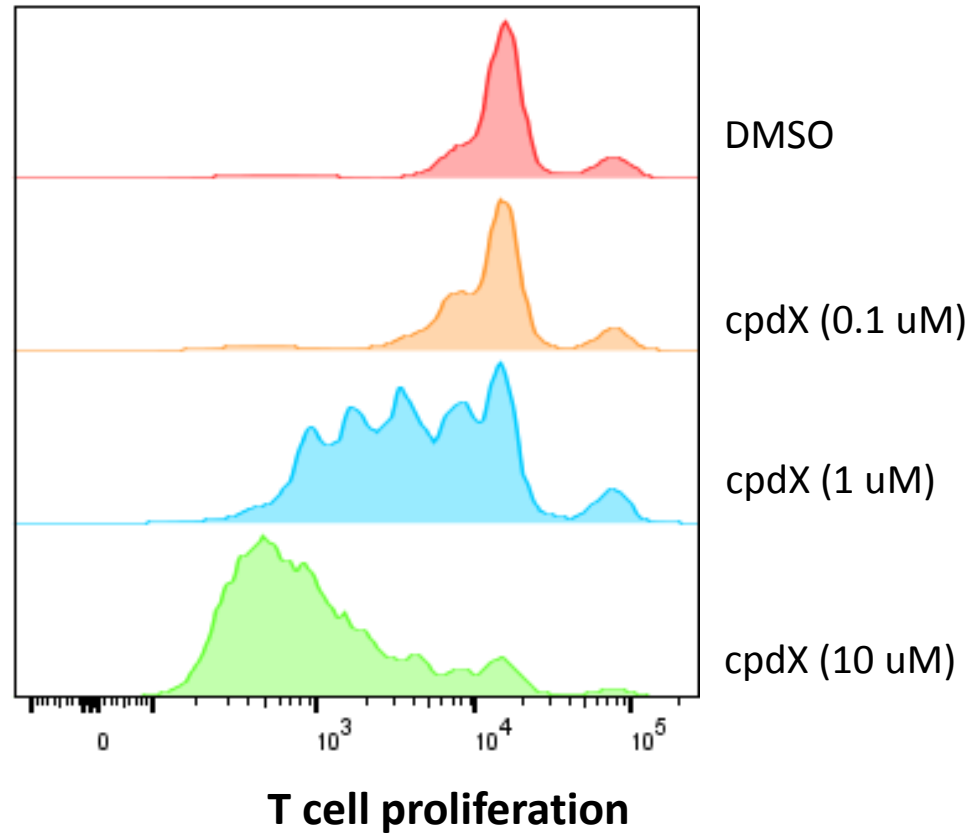
CO-culture with Teff



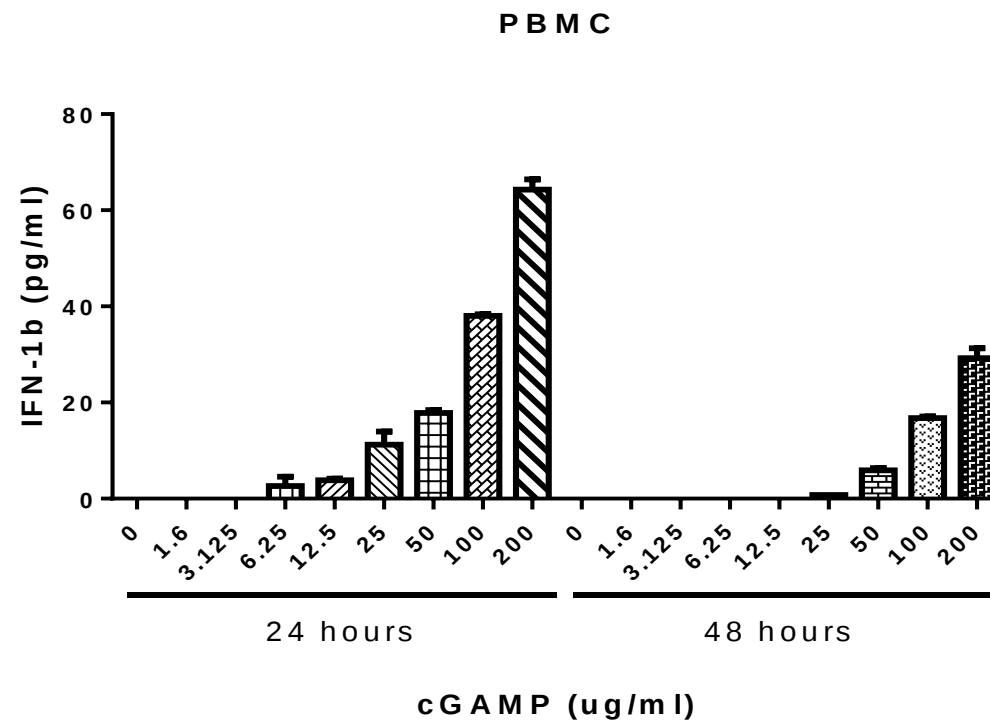
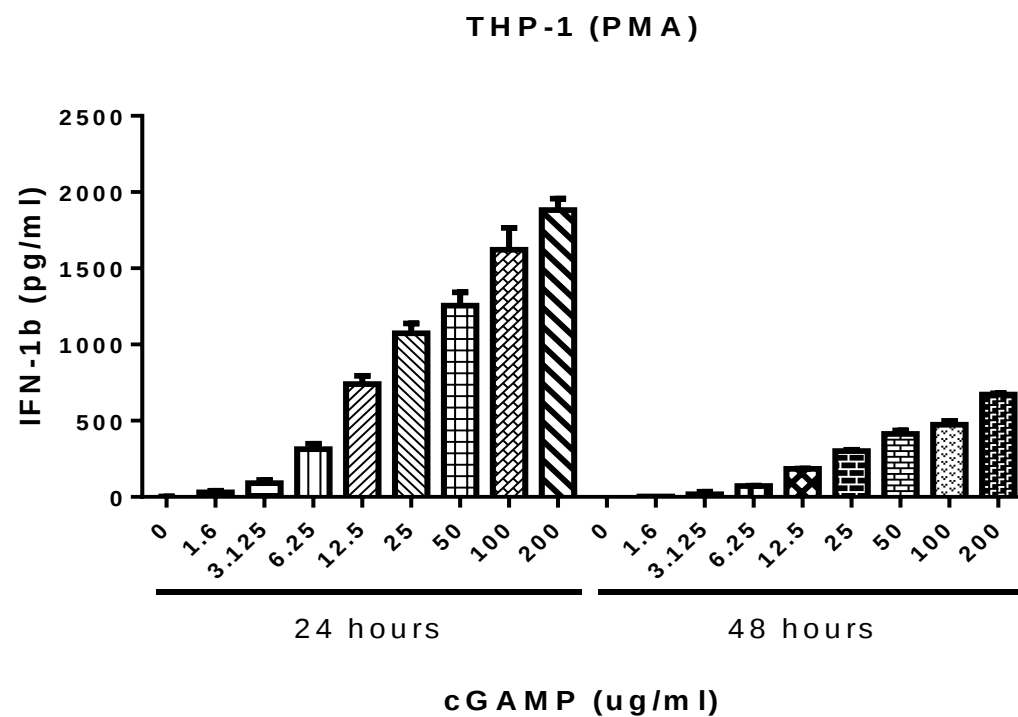
Tumor Cell Suppressive Assay

Co-culture with Immune Cells

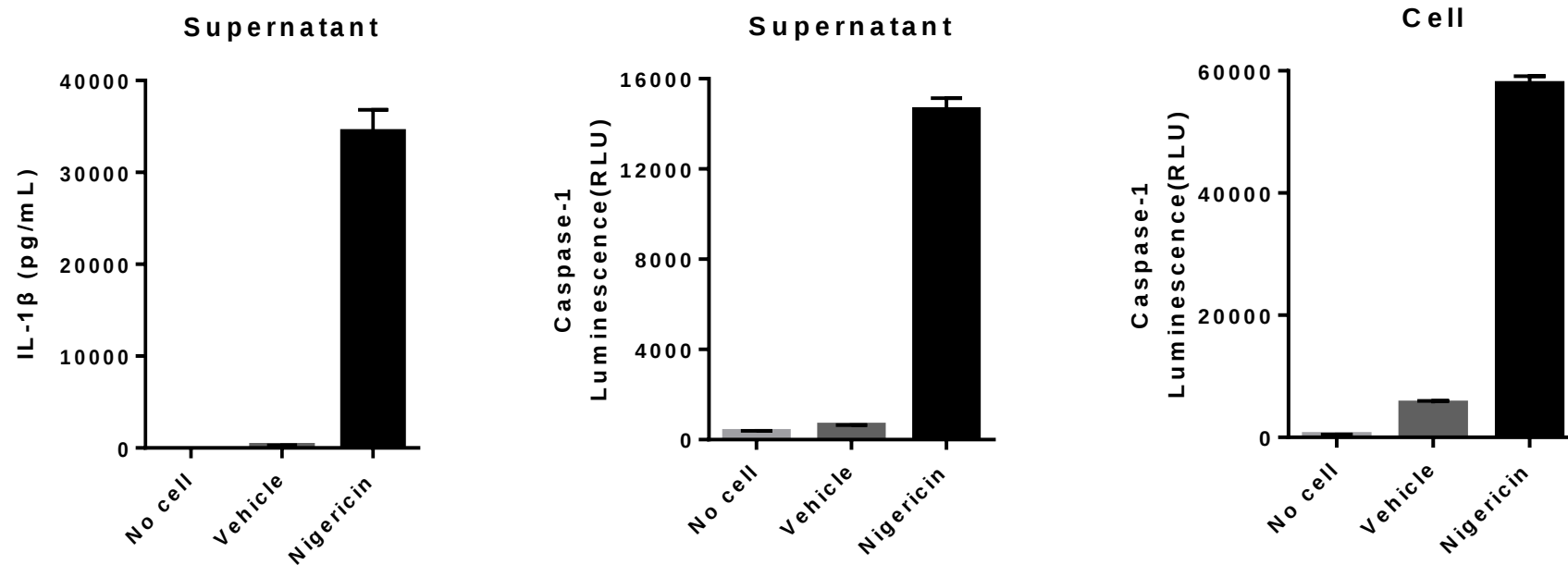
- Hela cell co-cultured with primary human T cells in the presence of cpdX.



STING Pathway Activation



- PMA-differentiated THP-1 cells were stimulated with LPS, followed by treatment with Nigericin for 1h





OUR COMMITMENT

Improving Health. Making a Difference.

For questions and requests, please email to info_onco@wuxiapptec.com



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