

Biogen welcomes

Melanie Villalobos Garcia

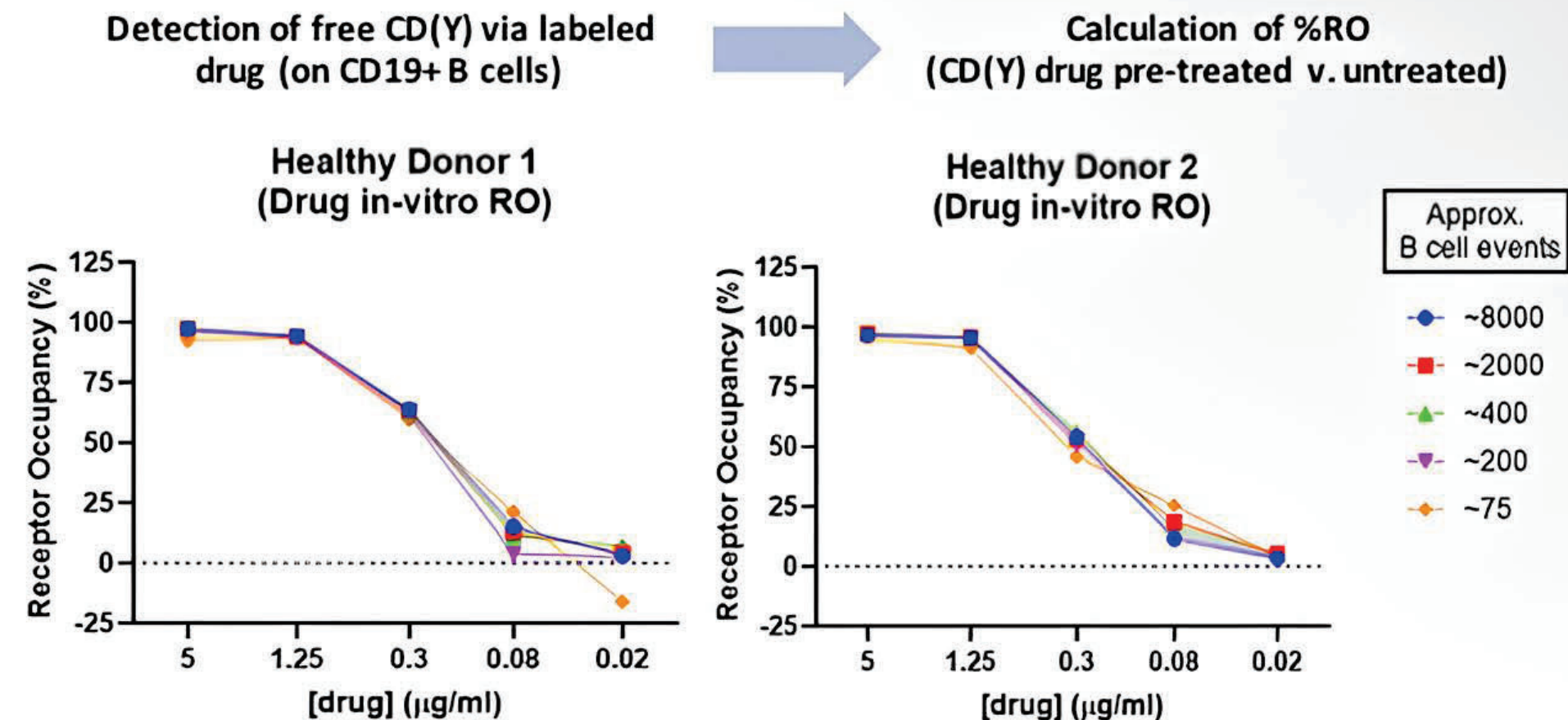
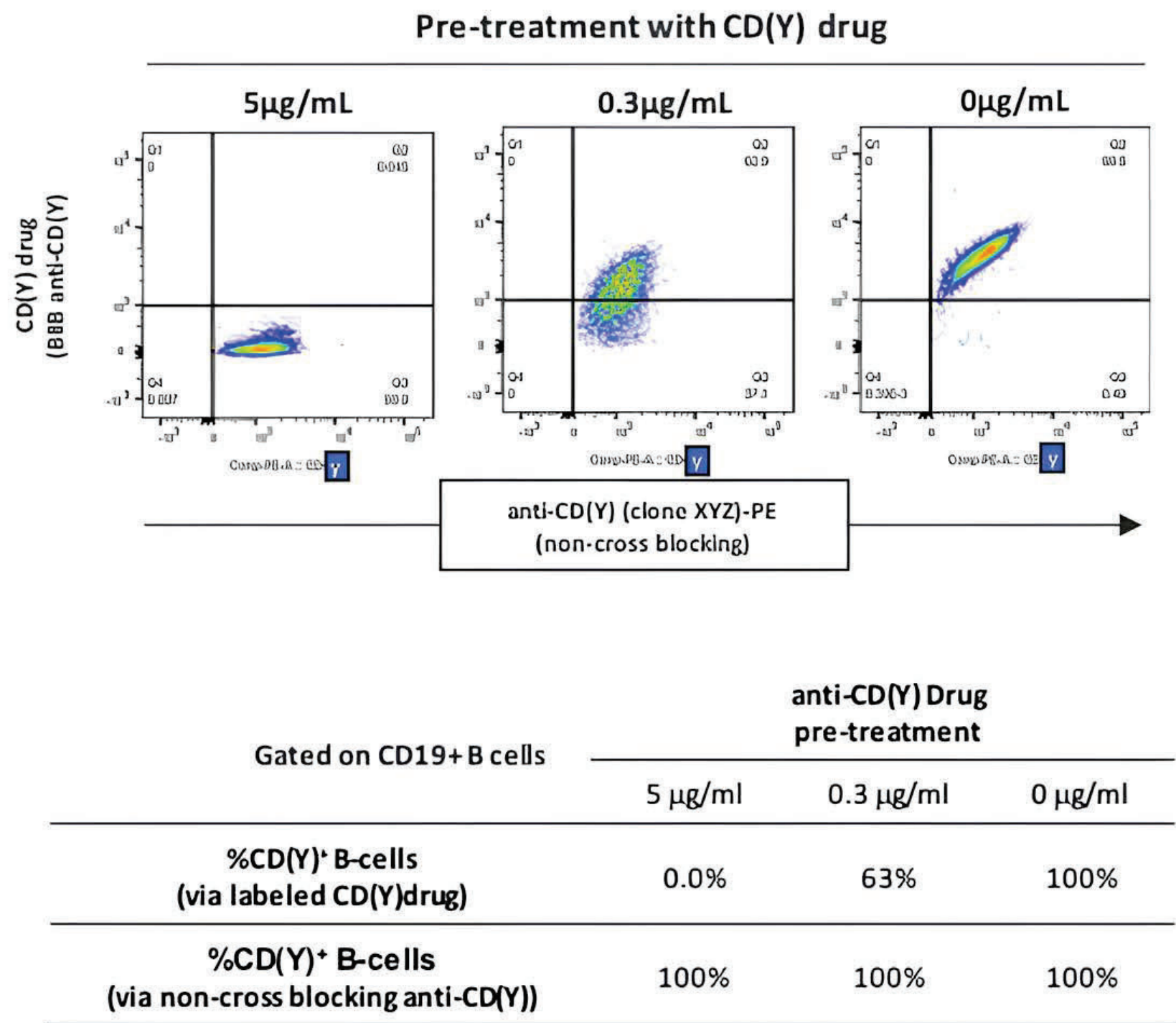
August 28th, 2023

co-op presentation #1 by Melanie Villalobos



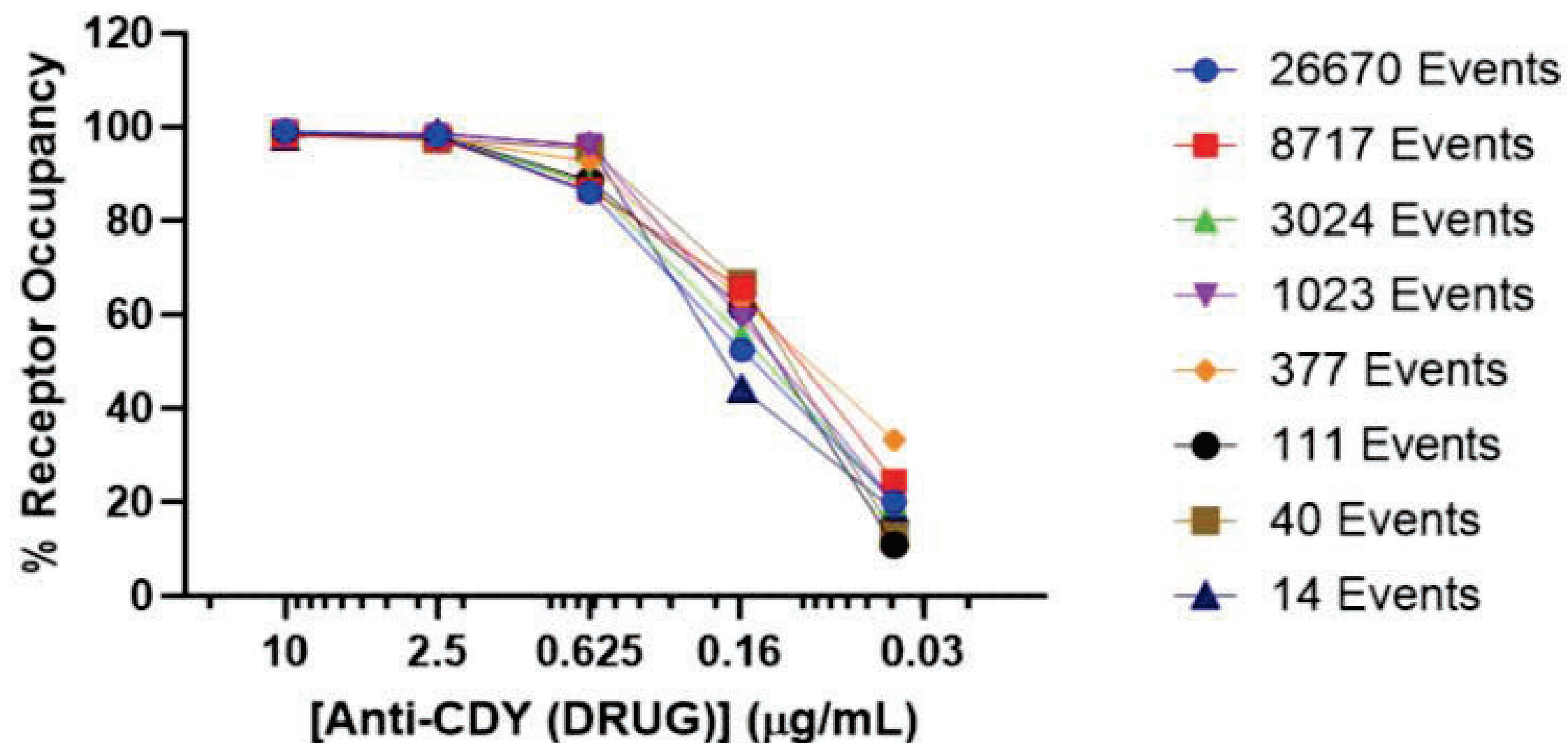
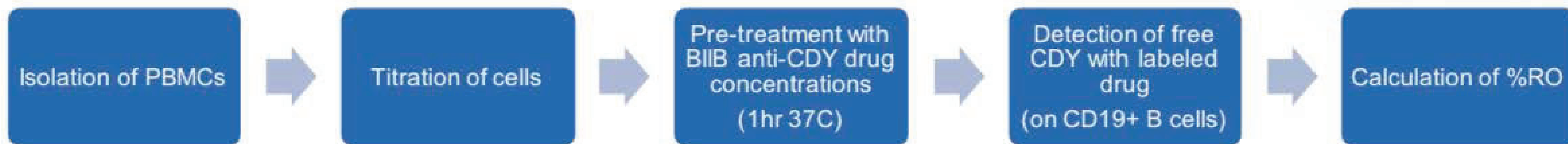
2

Cell Surface CD(Y) Receptor Occupancy | Use of a Flow Cytometry Assay



- Feasibility assessment of a novel flow cytometry method in CSF for measuring cell surface CD(Y) RO% suggests the potential for promising results.
- To be added to Final Presentation

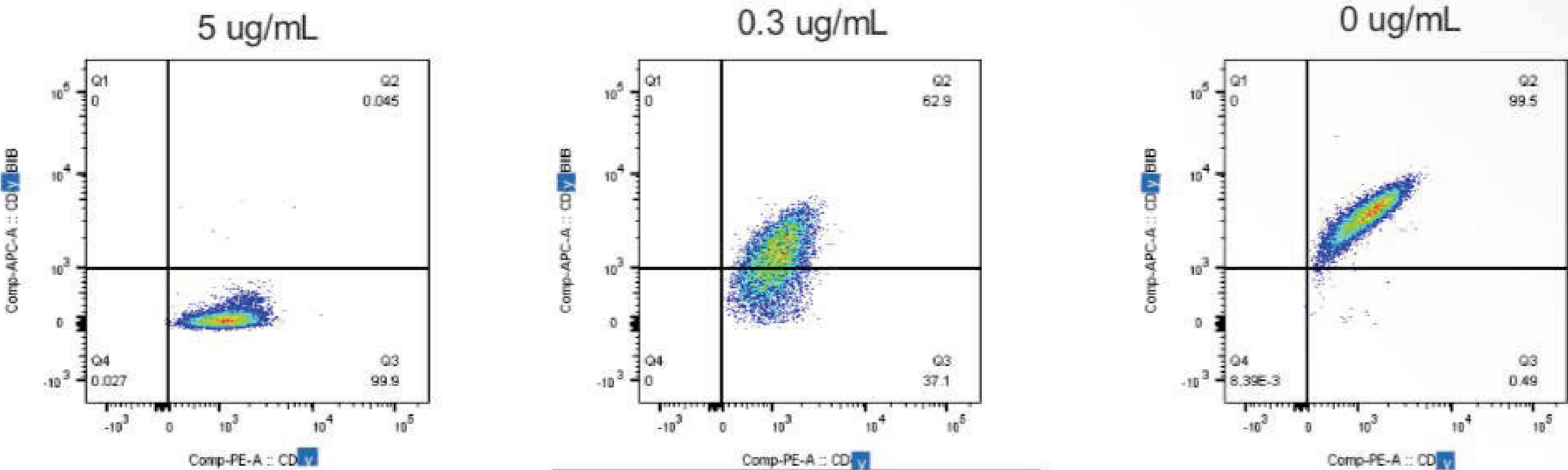
Mimicking target engagement in-vitro



CDY expression is not impacted by BIIB anti-CDY

BIIB anti-CDY pre-treatment

BIIB labeled anti-CDY



Non-cross blocking anti-CDY

	Pre-treatment with BIIB anti-CDY (unlabeled)		
	5 ug/ml	0.3 ug/ml	0 ug/ml
%CDY+ B cells based on labeled drug (adding Q1 & Q2)	0.0%	62.9%	99.5%
%CDY+ B cells based on non-cross blocking mAb (adding Q2 & Q3)	100%	100%	100%

Impact of cell surface drug fixation on target engagement

Isolation of PBMCs



Pre-treatment with BIIB anti-CDY drug



Fix v. Do Not Fix Drug on Cell Surface

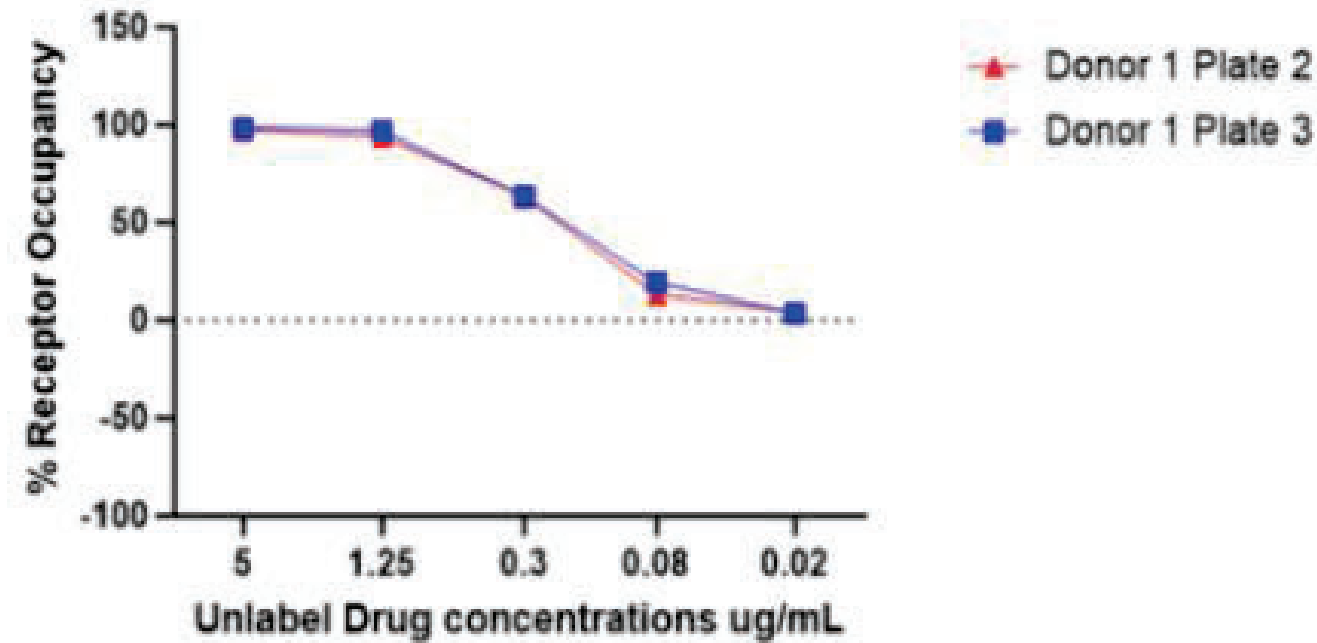


Titrate cell number

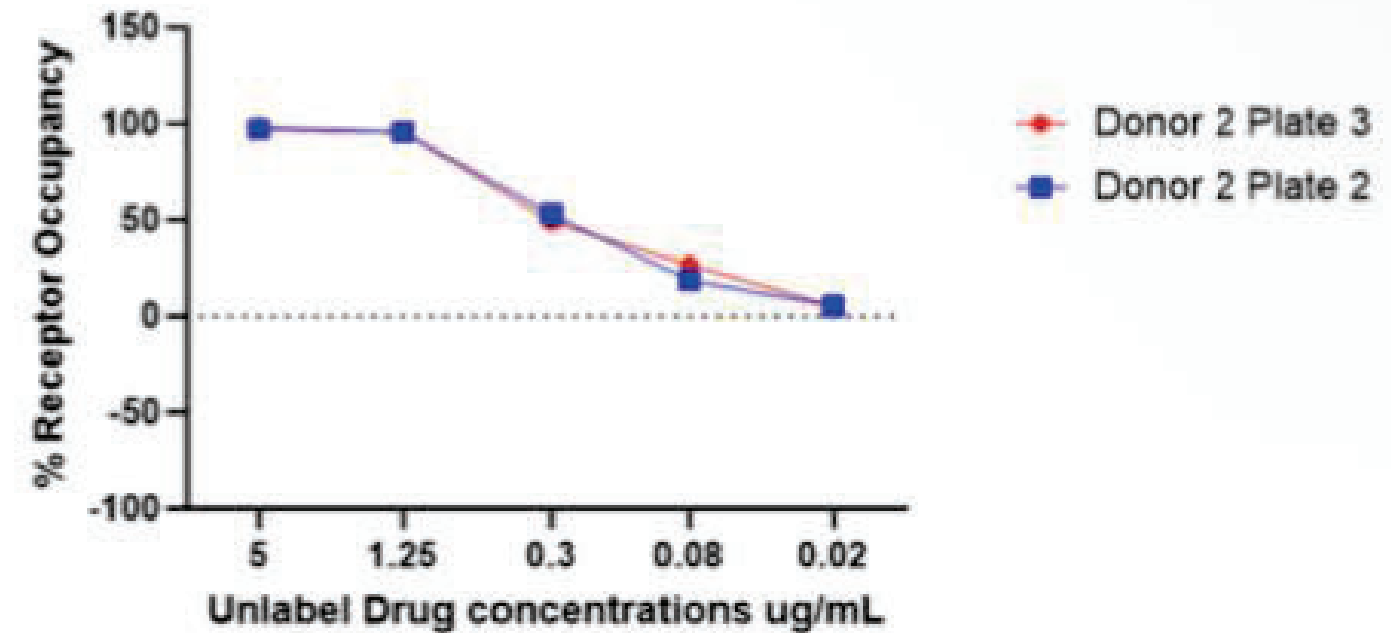


Detection of free CDY with labeled drug

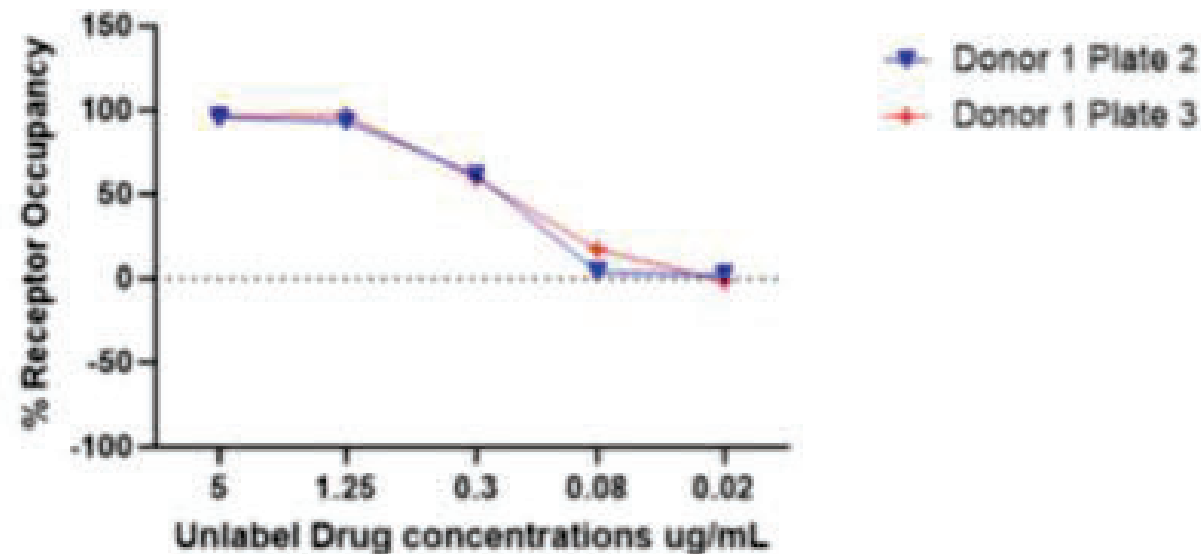
Fixed vs Not Fixed Target Occupancy for ~2000 events



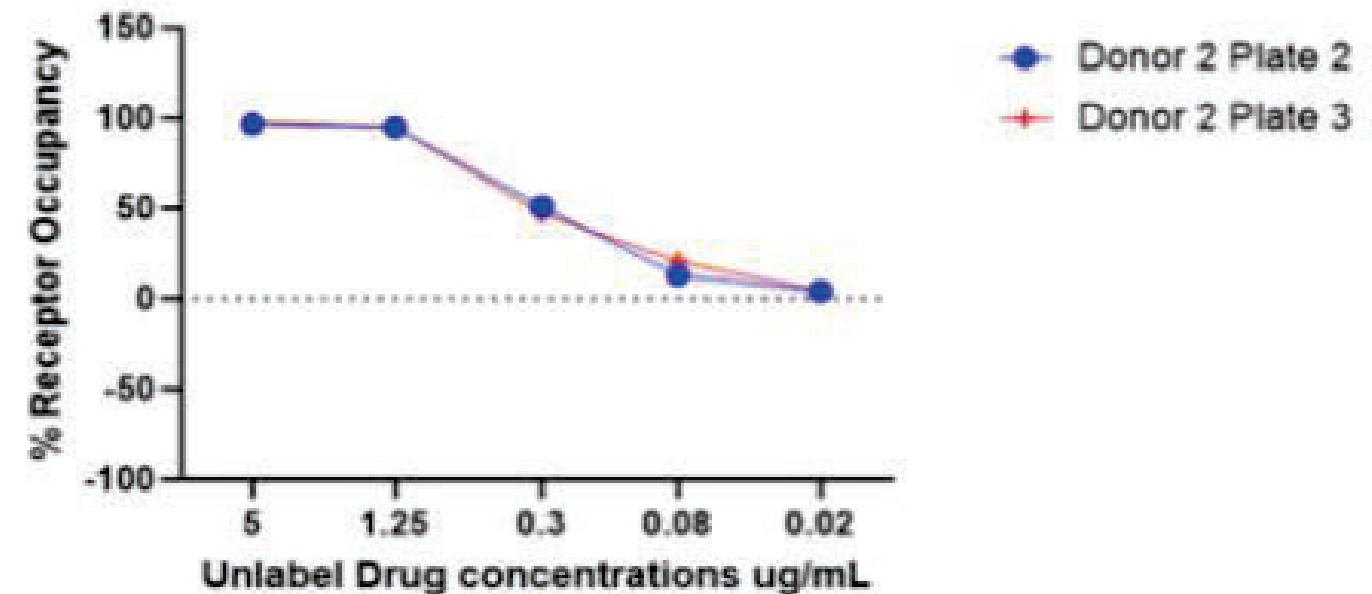
Fixed vs Not Fixed Target Occupancy for ~2000 events



Fixed vs Not Fixed Target Occupancy for ~180 events



Fixed vs Not Fixed Target Occupancy for ~180 events



Impact of drug fixation on target engagement

Isolation of PBMCs

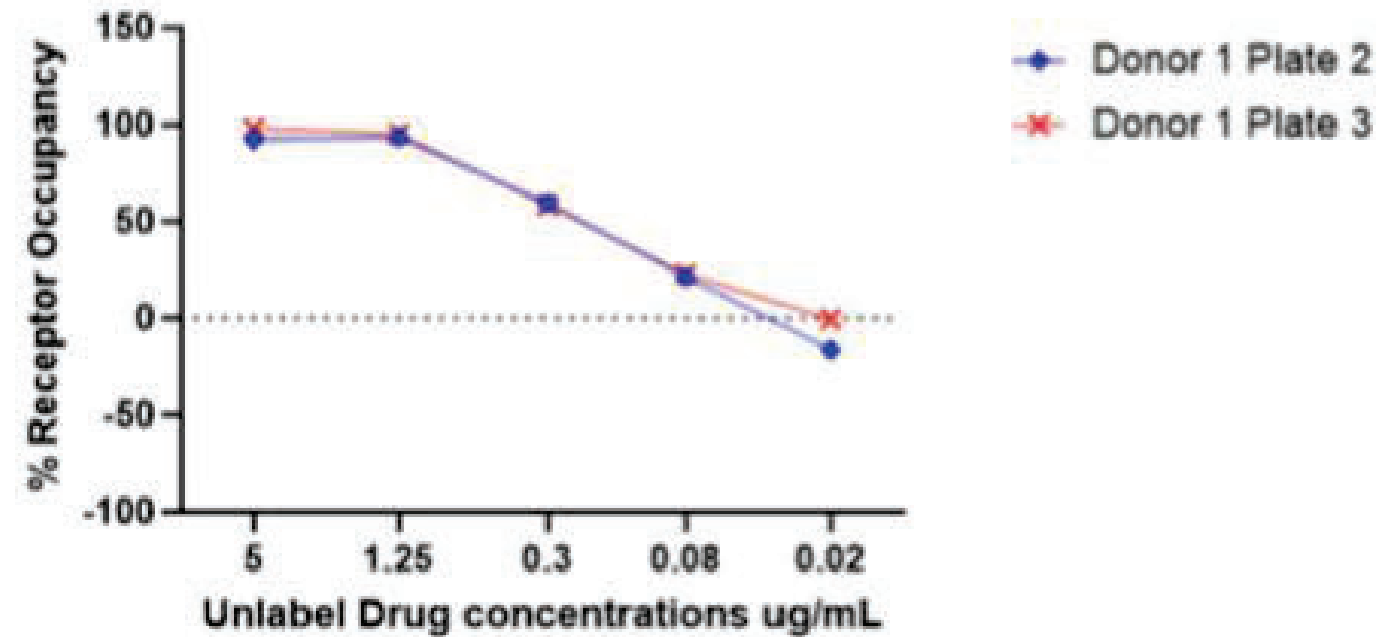
Pre-treatment with
BLIB anti-CDY drug

Fix v. Do Not Fix
Drug on Cell Surface

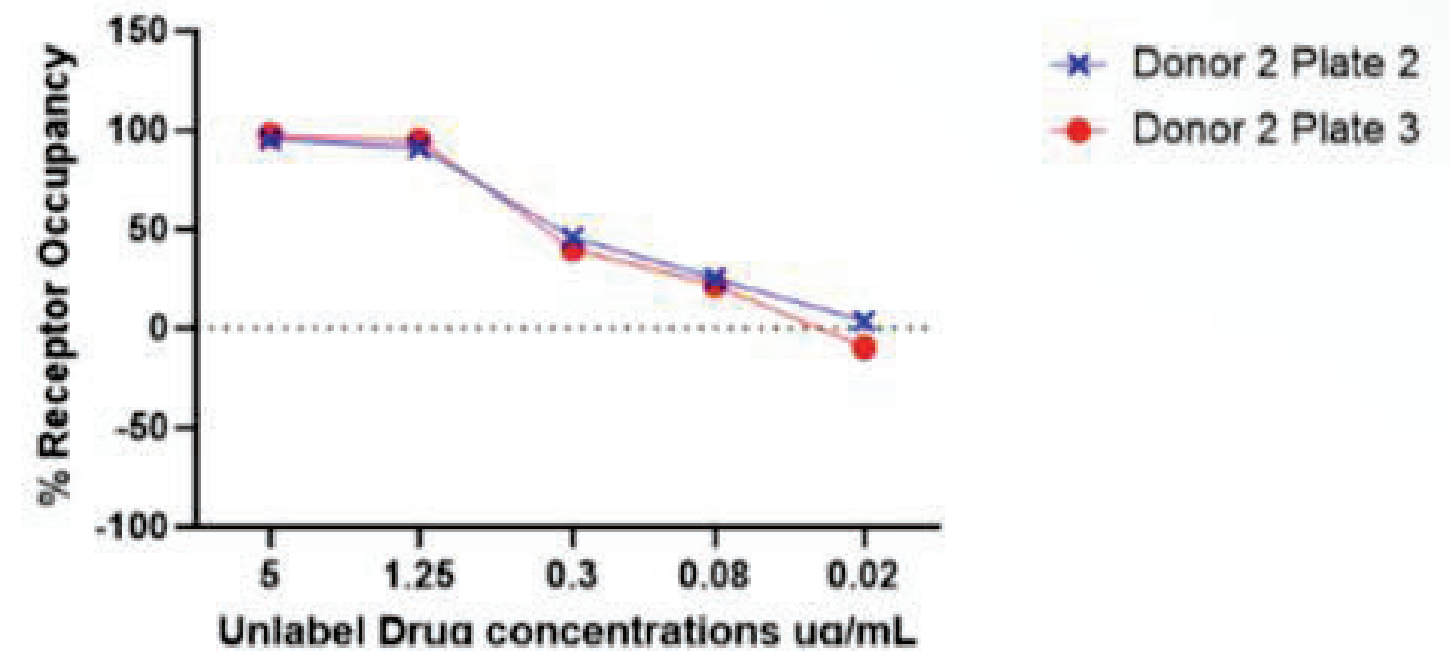
Titrate cell number

Detection of free
CDY with labeled
drug

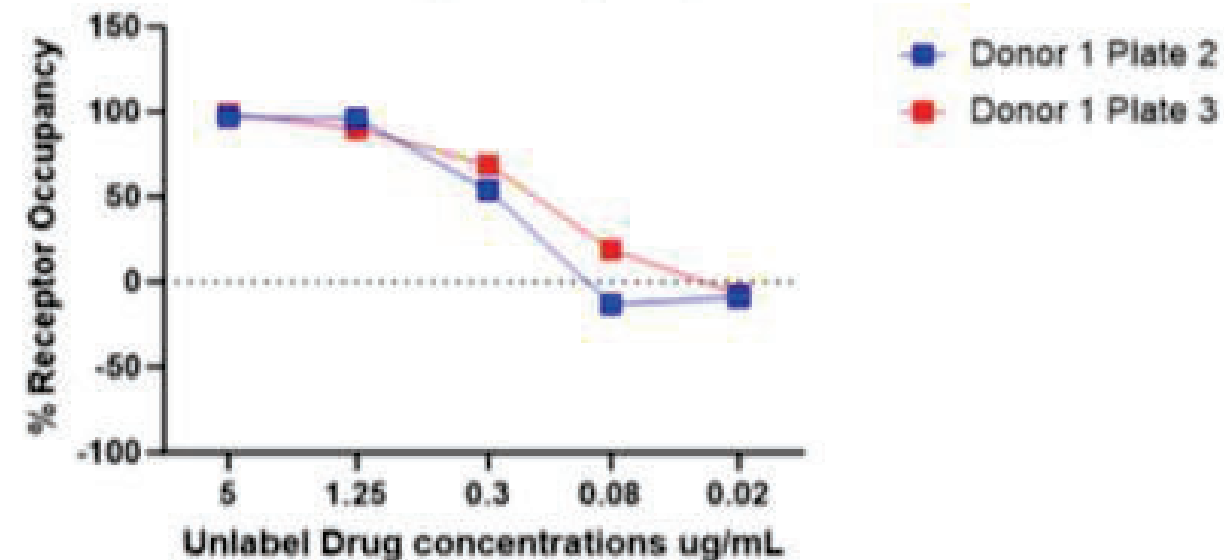
Fixed vs Not Fixed Target Occupancy for ~70 events



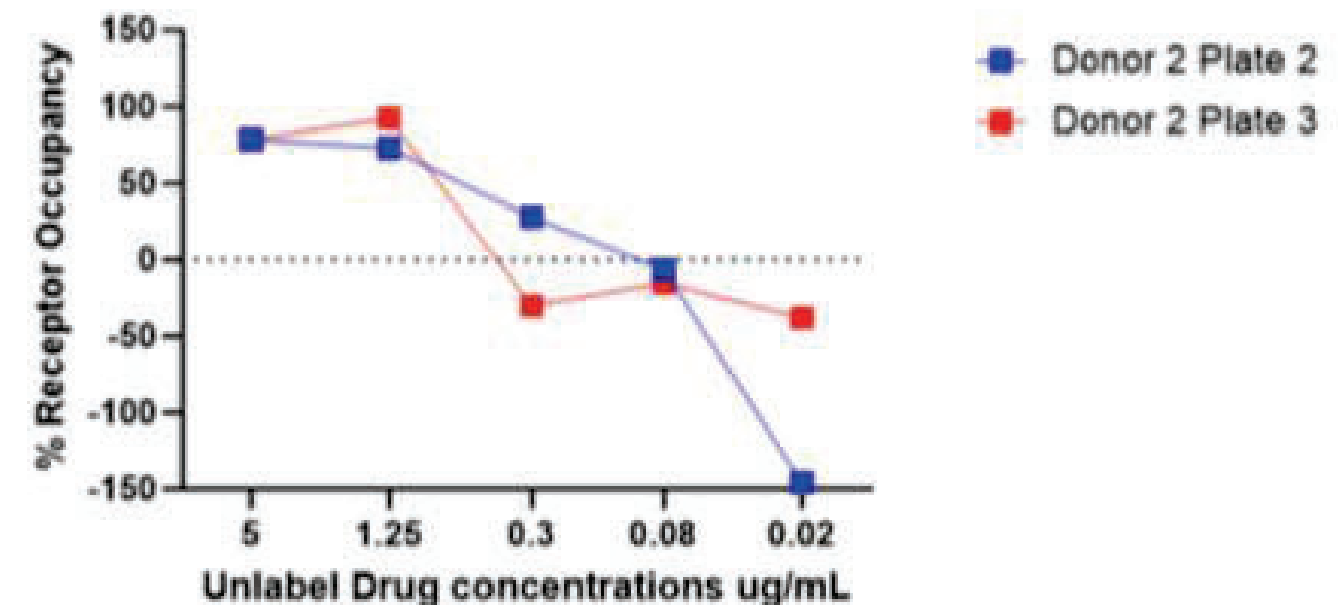
Fixed vs Not Fixed Target Occupancy for ~70 events



Fixed vs Not Fixed Target Occupancy for ~8 events



Fixed vs Not Fixed Target Occupancy for ~8 events



Target engagement is consistent across various cell numbers

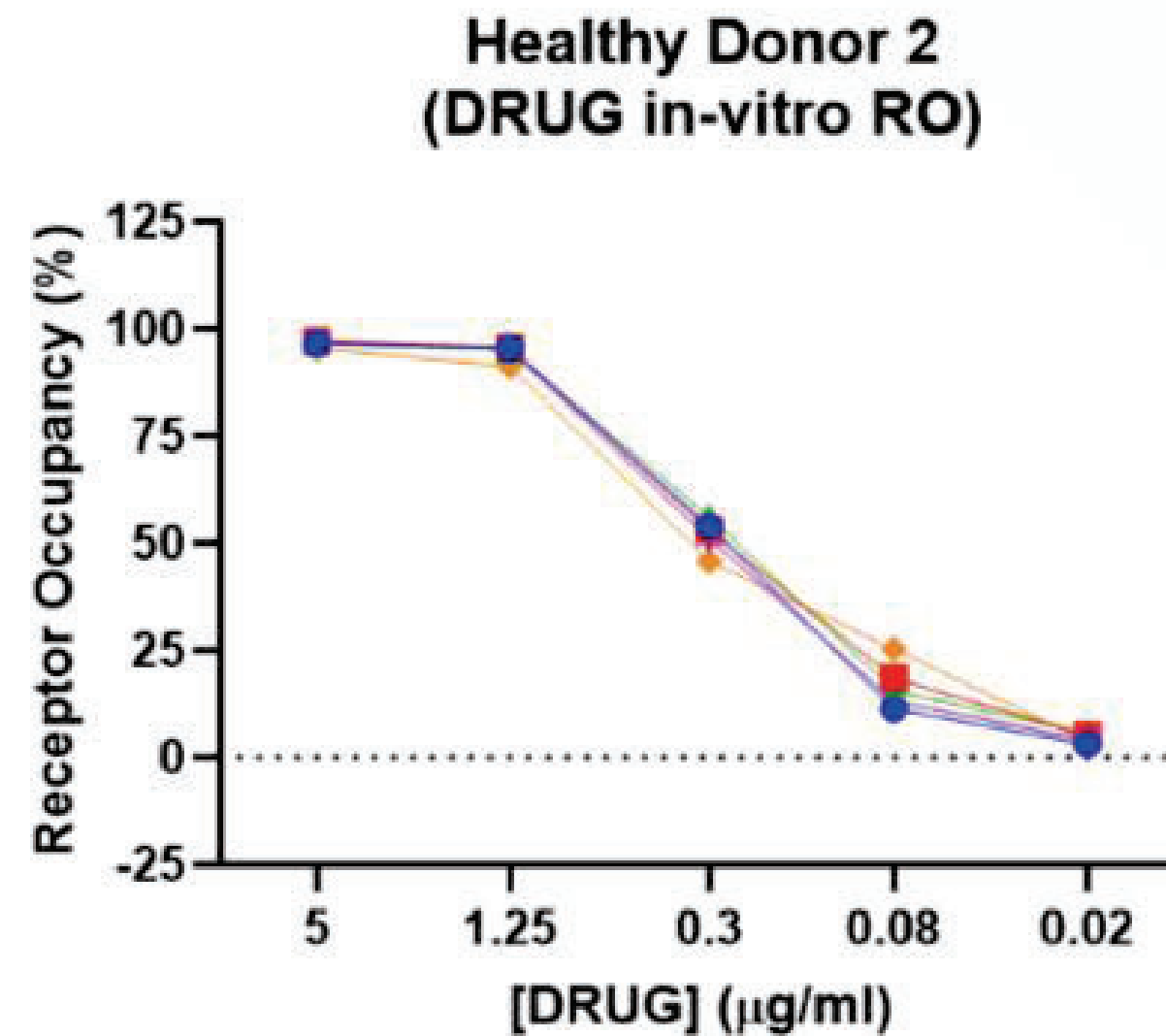
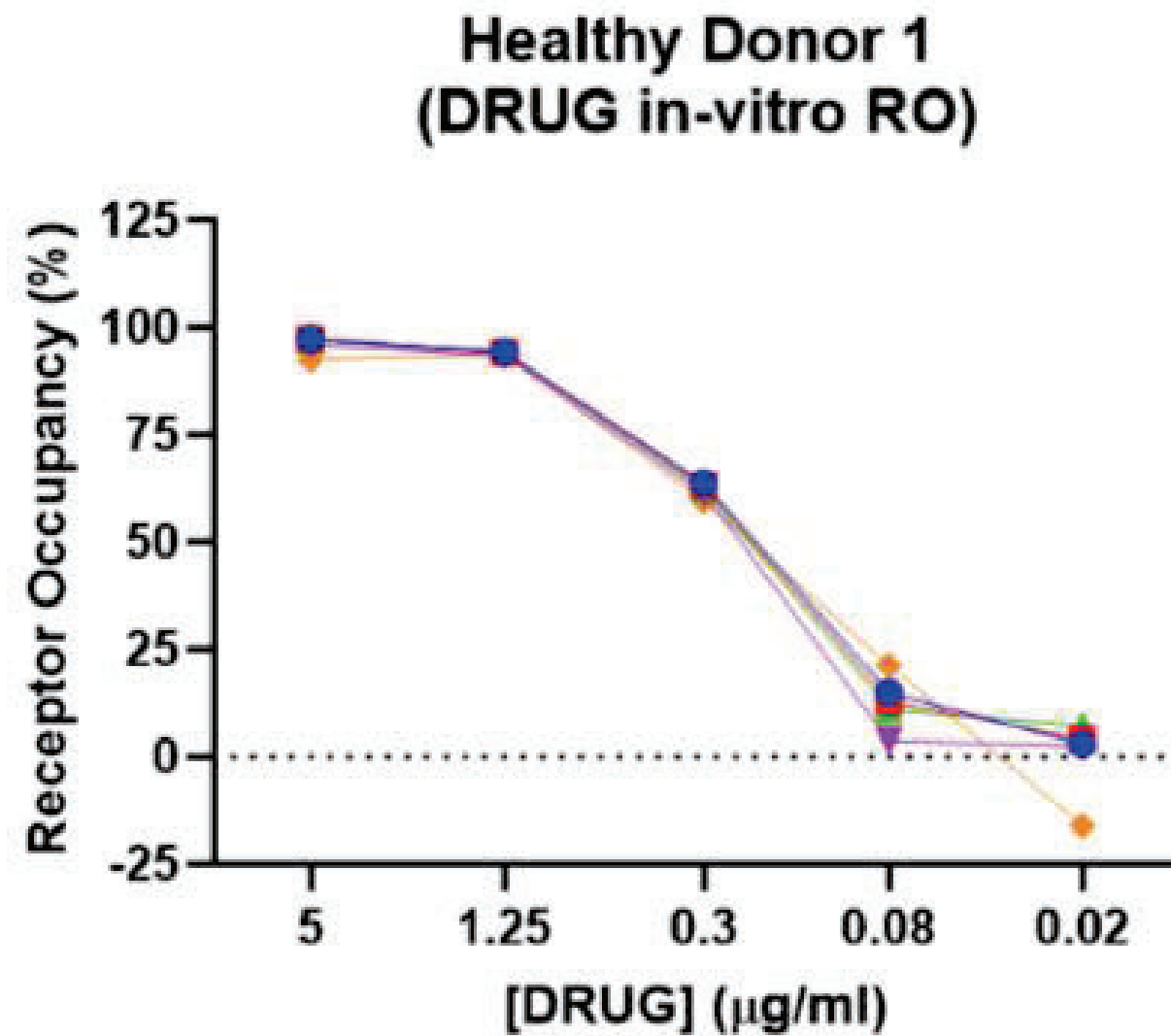
Isolation of PBMCs

Pre-treatment with
BIB anti-CDY drug

Fix v. Do Not Fix
Drug on Cell Surface

Titrate cell number

Detection of free
CDY with labeled
drug



Approx.
B cell events

- 8000
- 2000
- 400
- 200
- 75

Target engagement between donors

Isolation of PBMCs



Pre-treatment with
BIB anti-CDY drug



Fix v. Do Not Fix
Drug on Cell Surface



Titrate cell number



Detection of free
CDY with labeled
drug

Plate 2 "Not Fixed" Target Occupancy for ~2154.6 events

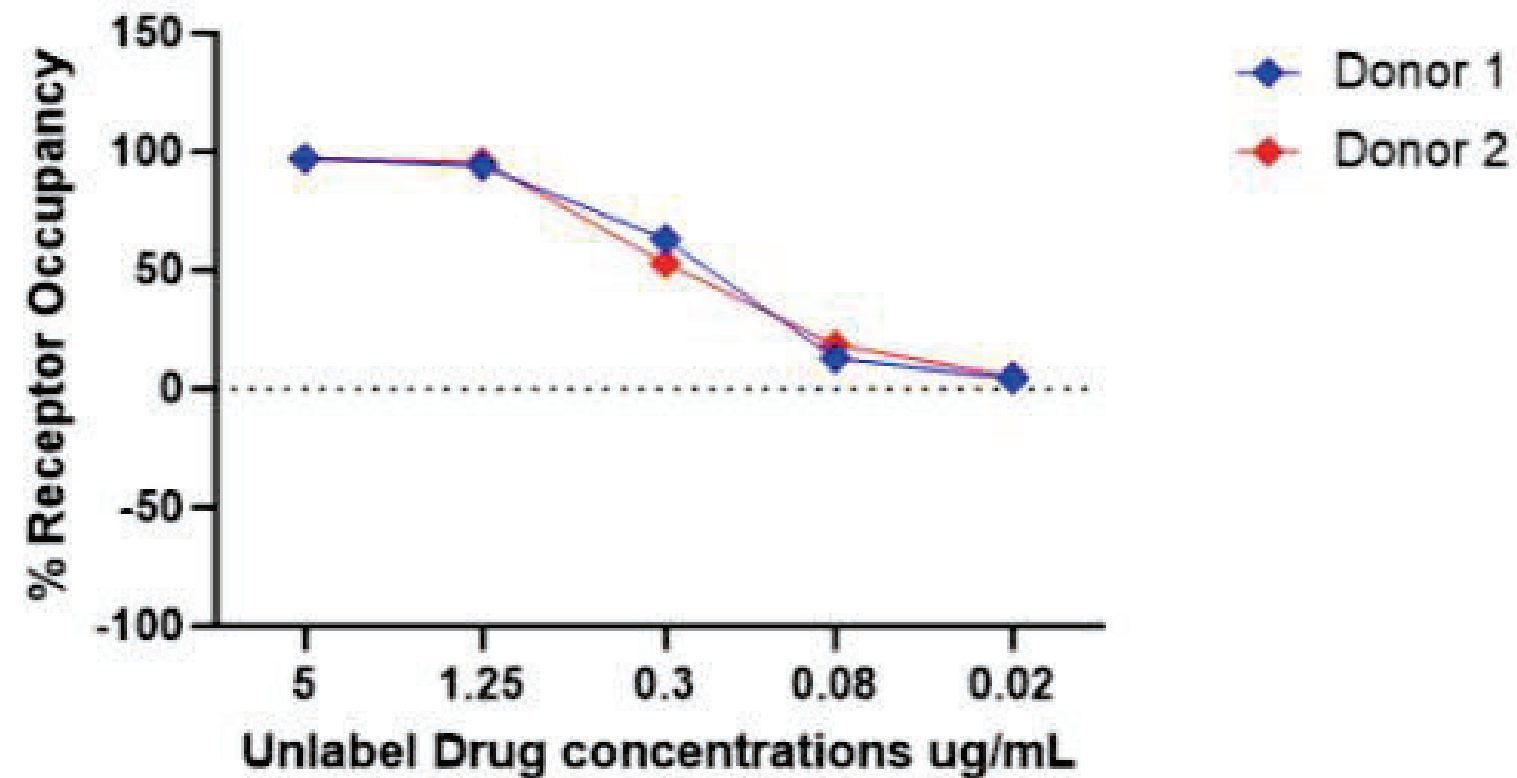
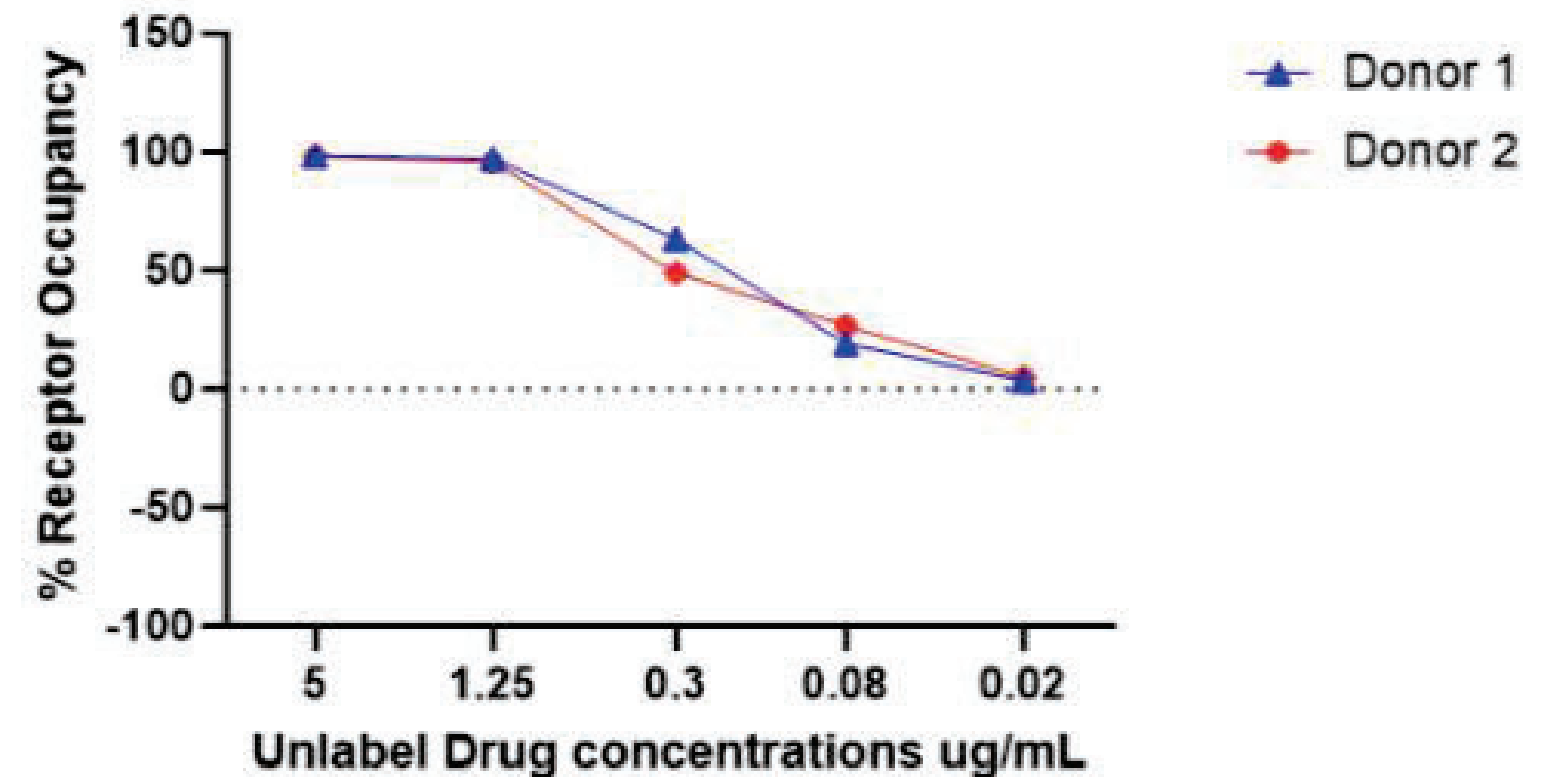


Plate 3 "Fixed" Target Occupancy for ~1691 events



Next steps

- Increase N of donors to understand variability in TE at lower drug concentrations and lower cell counts to increase confidence on CSF feasibility
- Assess, cell viability and TE in cells resuspended in artificial CSF
- Test transfix tubes, designed to collect cells from CSF in fixative solution.
- Assess, cell viability and TE in patient CSF (data for oct 1st presentation)