Development of a multi-parameter flow cytometric assay for the detection of primed T cells

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Standard methodologies

Elispot/ ICC

- **Short term assays:detect immediate cytokine production**
- **good at detecting effector cells but perhaps not central memory cells**

Proliferation assays

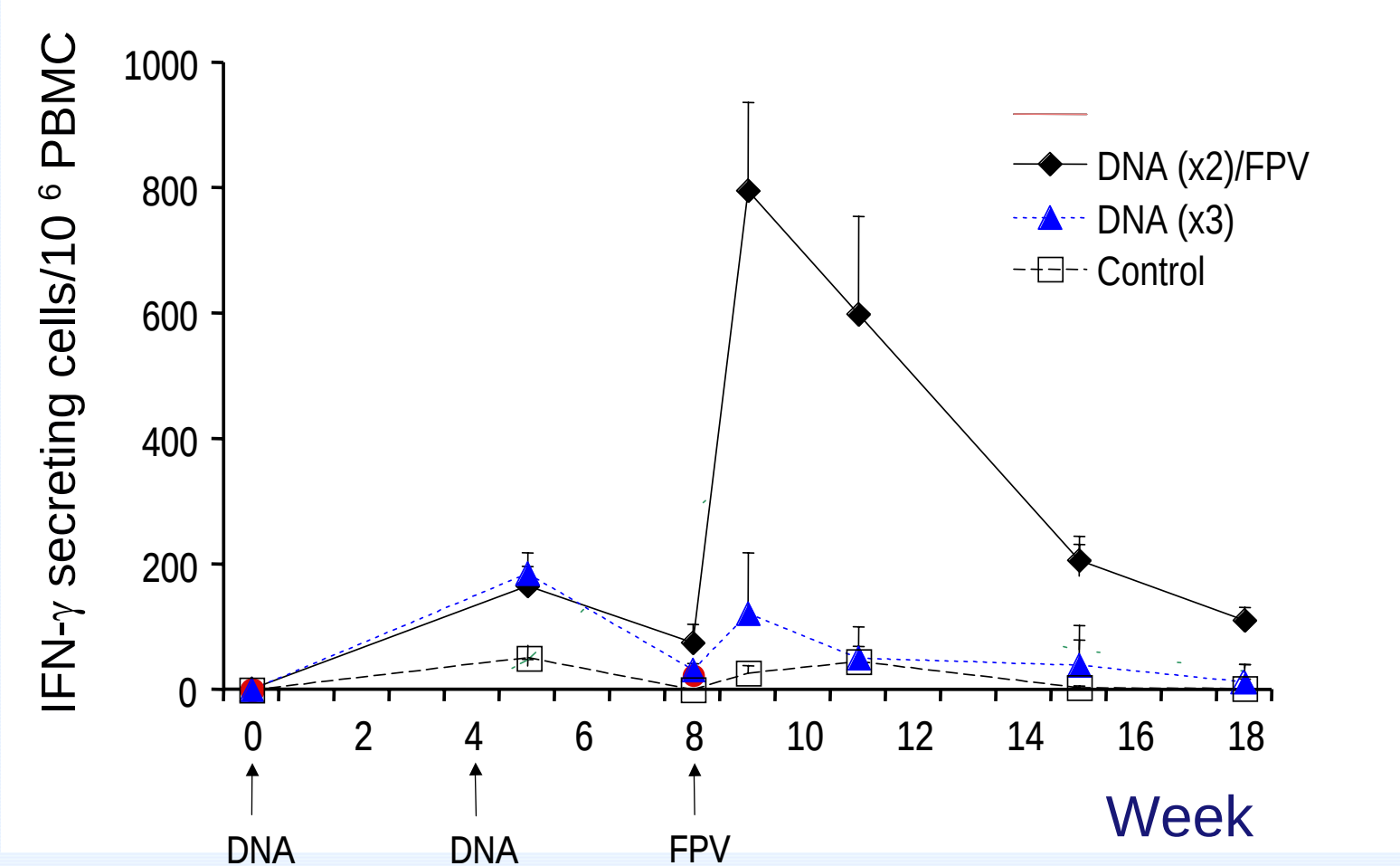
- **ability of cells to proliferate, but no insight into function or phenotype**
- **CFSE labeling allows delineation of cell type proliferating**

Objectives

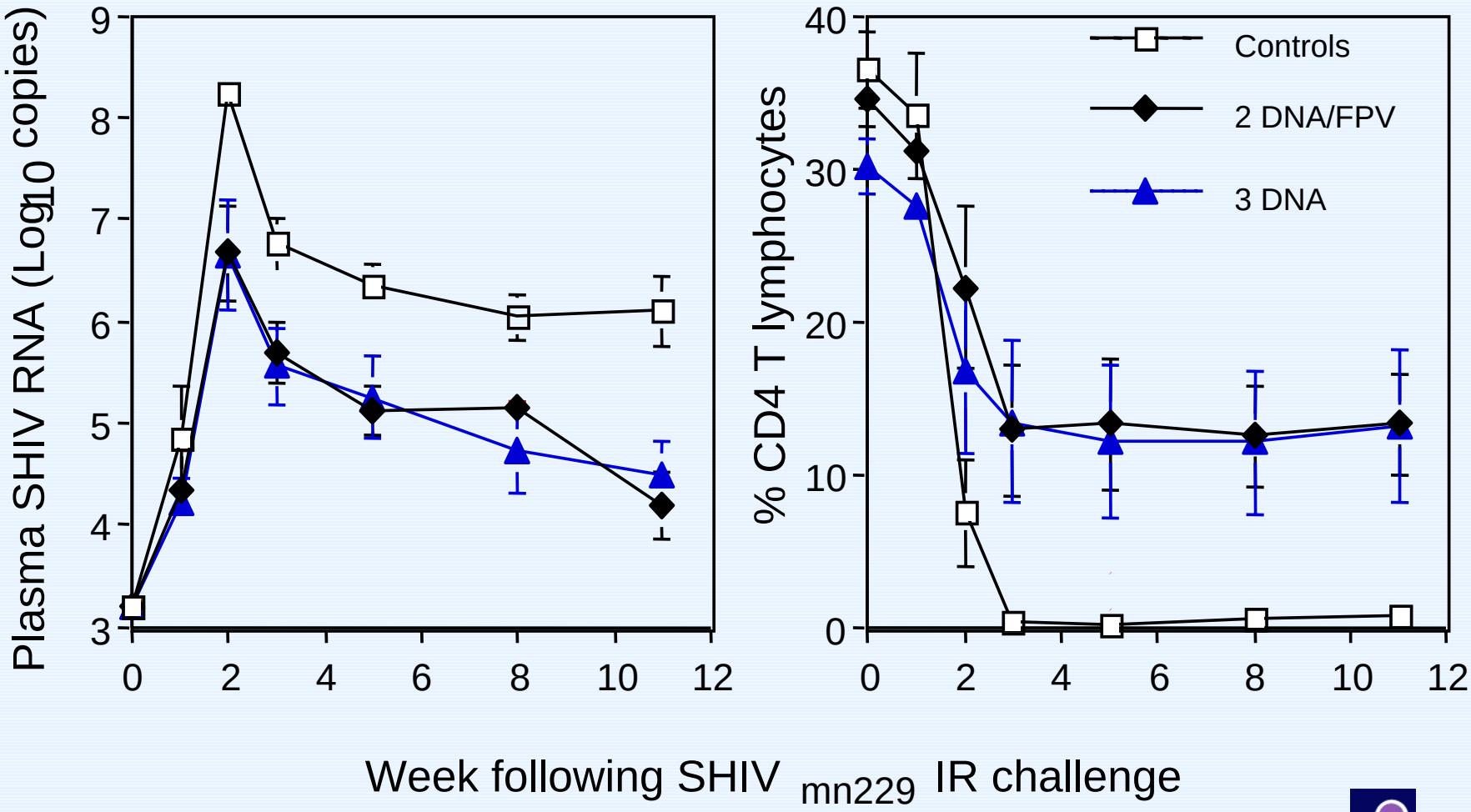
- **Develop assay that allows detection of small numbers of primed or quiescent cells**
- **Assay should provide insight into function and phenotype**
- **It should be relatively simple to set up with relatively little *in vitro* manipulation**

Why?

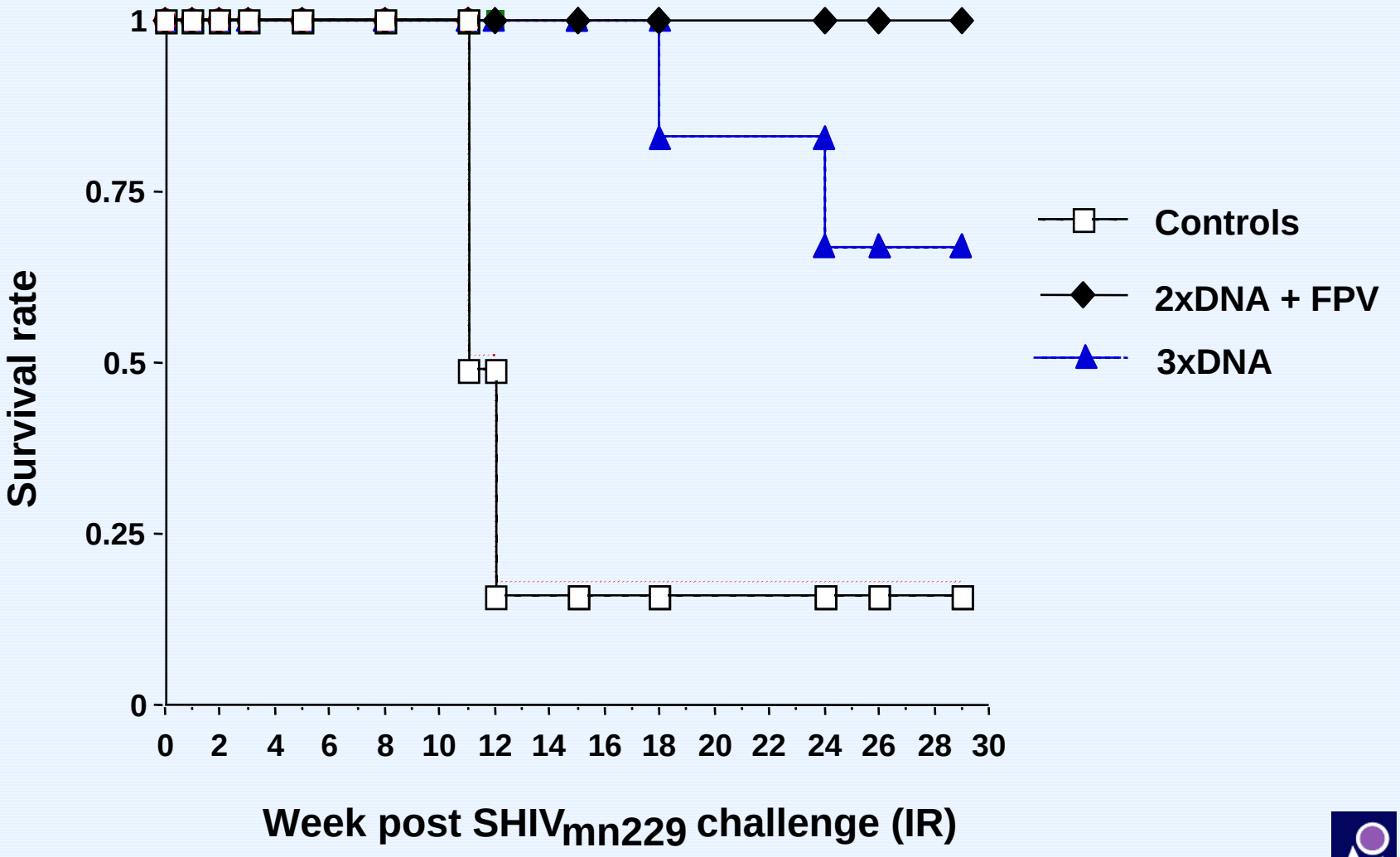
SIV Gag-specific Response by IFNγ ELISPOT

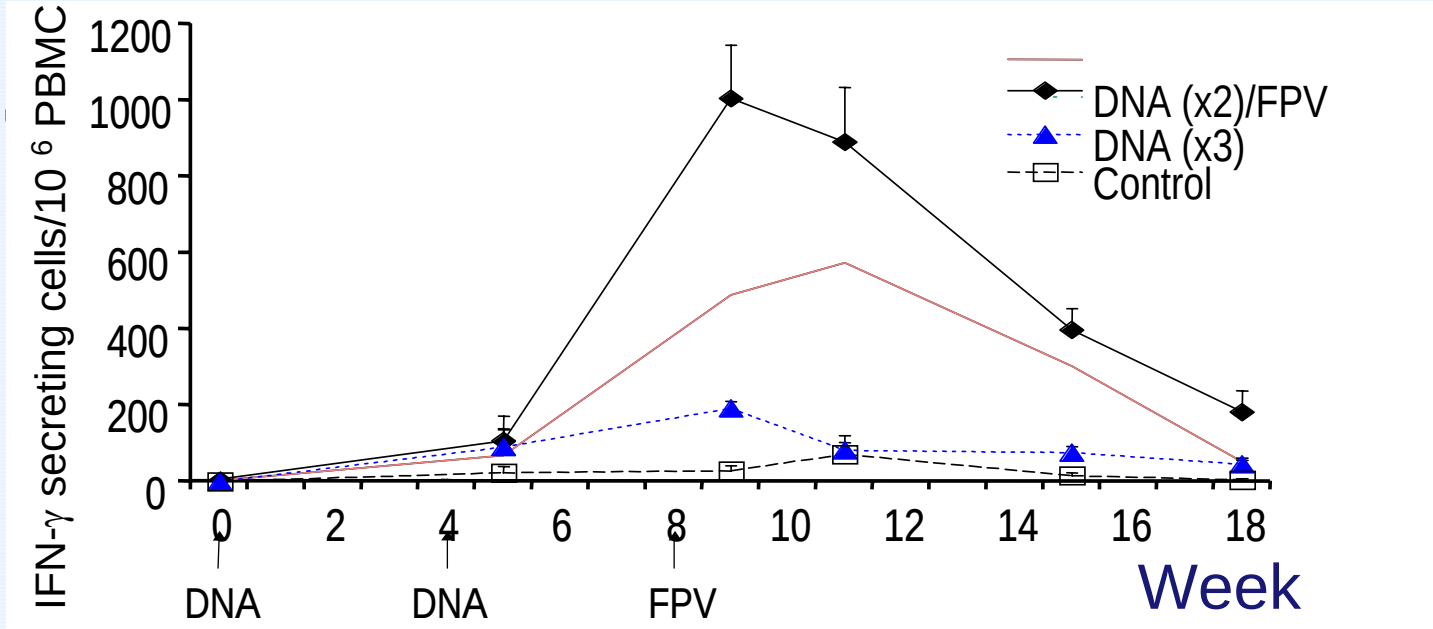


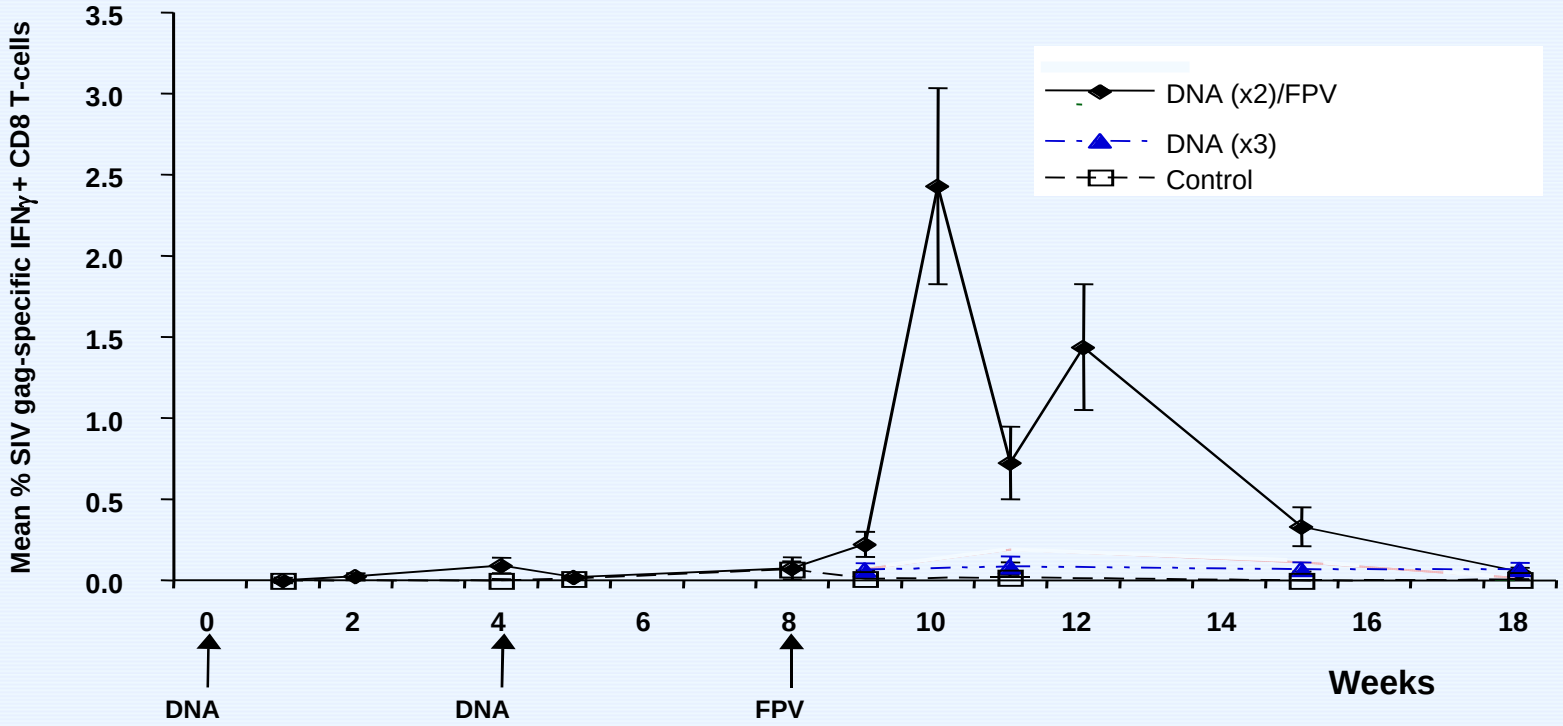
Control of pathogenic SHIV challenge by DNA/FPV prime/boost immunisation



Survival of monkeys post challenge




IFN-γ secreting cells/10 6 PBMC
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
18
DNA
DNA
FPV
Week
DNA (x2)/FPV
DNA (x3)
Control


Mean % SIV gag-specific IFNγ+ CD8 T-cells
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
12
14
16
18
DNA
DNA
FPV
Weeks
DNA (x2)/FPV
DNA (x3)
Control

An assay to unmask priming

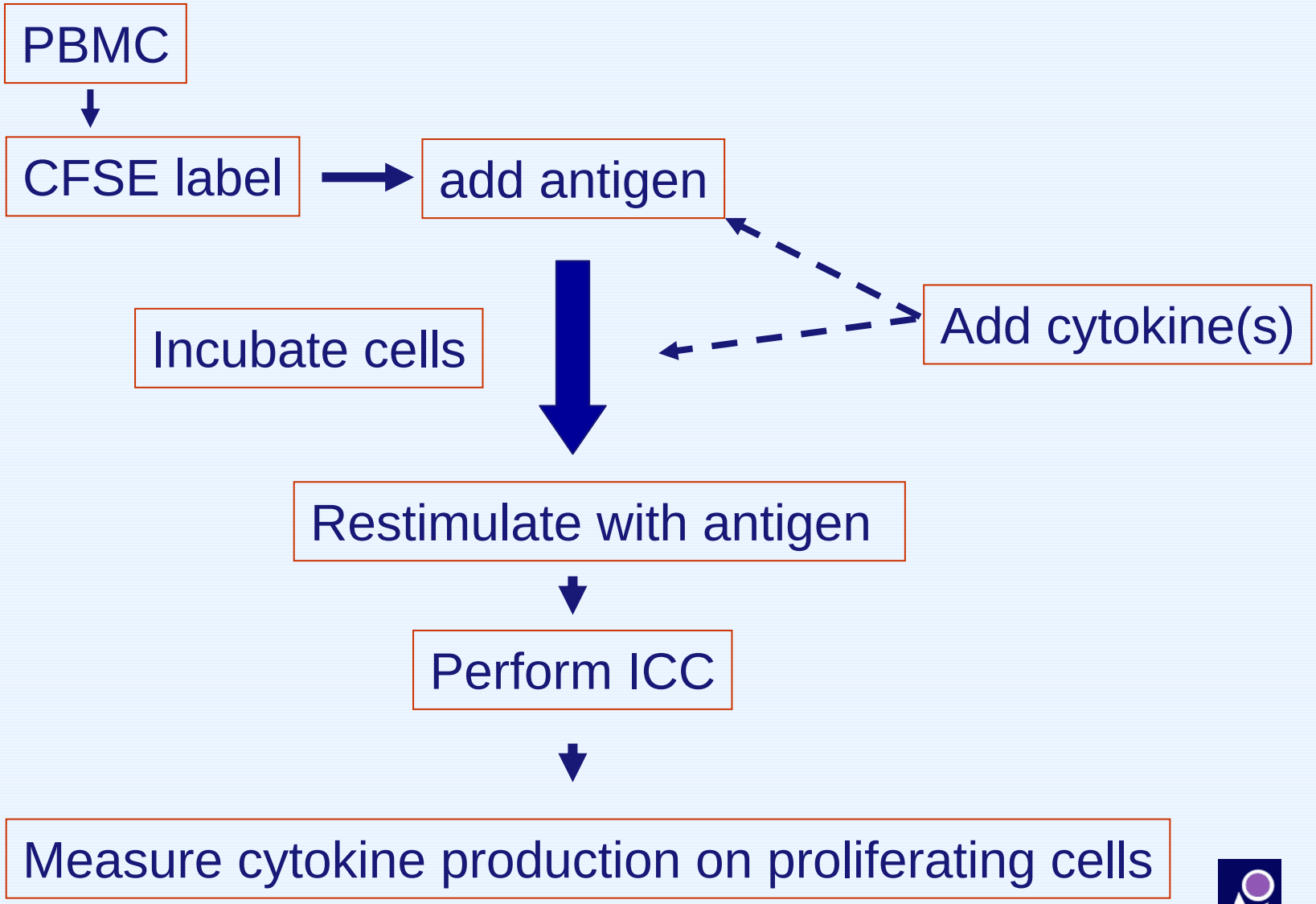
- **Combination of 2 Robust methods:**
  - **CFSE staining to show proliferation**
  - **ICC assay to detect Ag specific responses (cytokine)**

- **Addition of cytokine to boost small responses**
  - **IL-2, IL-7, IL-15:**
    - **biological characteristics somewhat similar**
      - **act as T cell growth and survival factors**
      - **share the common cytokine receptor gamma chain (CD132)**
      - **implicated in memory cell generation**
      - **produced by different cells**

- **c/w**

Amplispot	**Nixon lab**	**Effector cells**
Cultured Elispot:	**Hill lab**	**Non amplified**

Basic proposed methodology



Parameters to be considered in initial work-up

- With and Without Cytokine:
  - IL-2, IL-7, IL-15,
- Varying Concentrations of Cytokine
  - (IL-2, IL-7, IL-15 & IL-7+ IL-15):
- Combinations of cytokine
- Length of incubation
- Varying time of cytokine addition:
  - day 0 vs day 3 vs day 5
- Gating strategies

Other issues

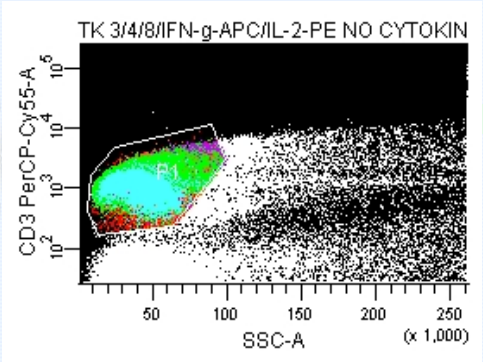
- Optimal antigen

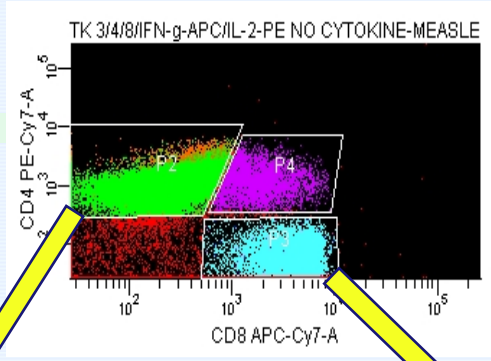
  CMV-lysate/CEF

  Measles

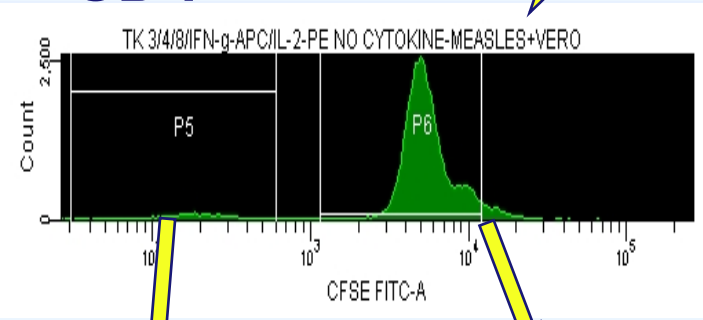
  Vaccinia
- Frozen PBMCs
- 24 vs 96 well plate
- Retrieval of cells post EDTA

Assay Gating

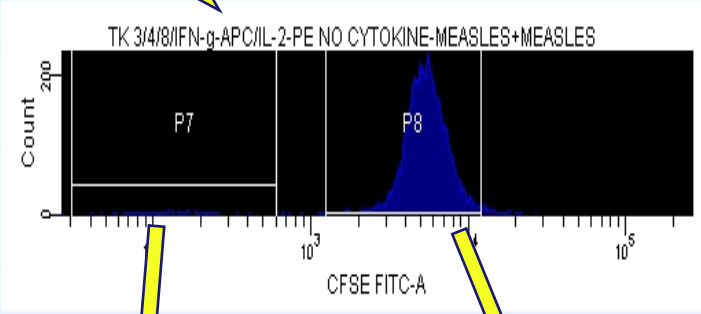




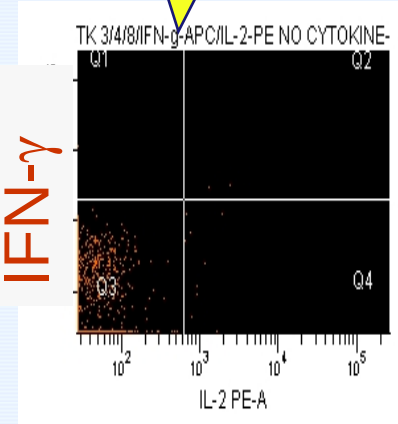
CD4+

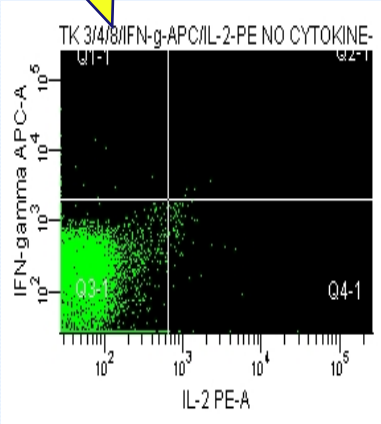


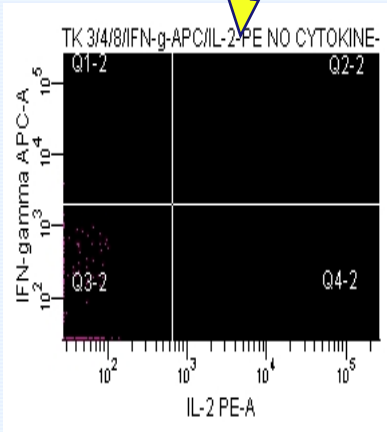
CD8+

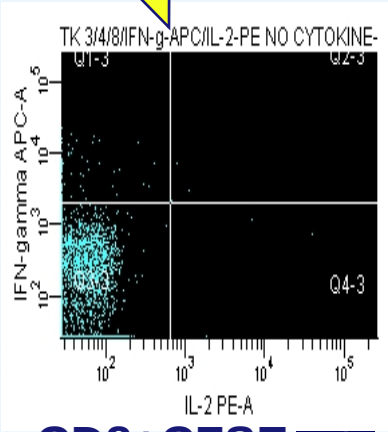


IFN-γ









CD4+CFSE dim

CD4+CFSE bright

IL-2

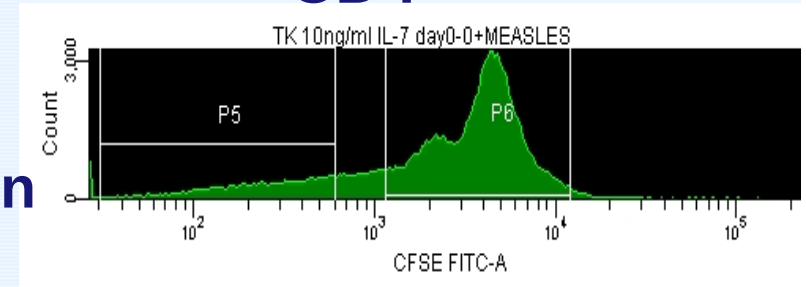
CD8+CFSE dim

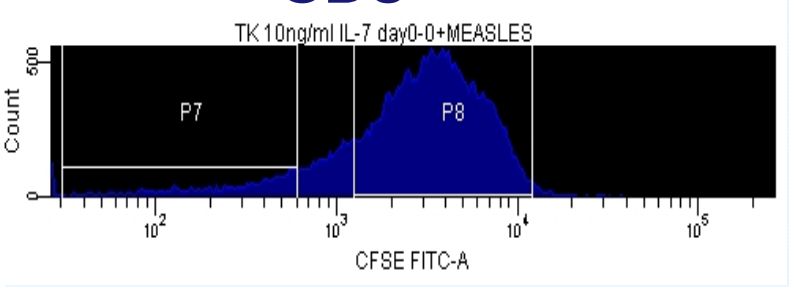
CD8+CFSE bright

Comparison of CD4 & CD8 responses to using 10 ng/ml IL-7 added at Day 0

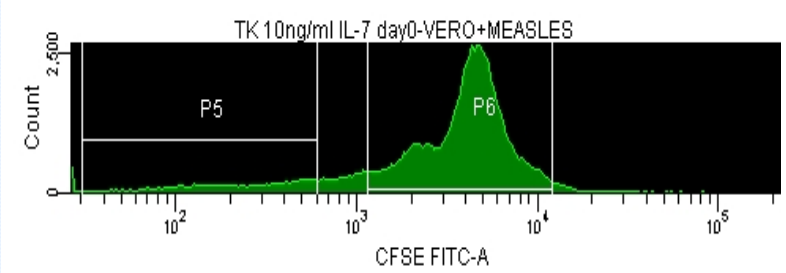
CD4+ **CD8+**

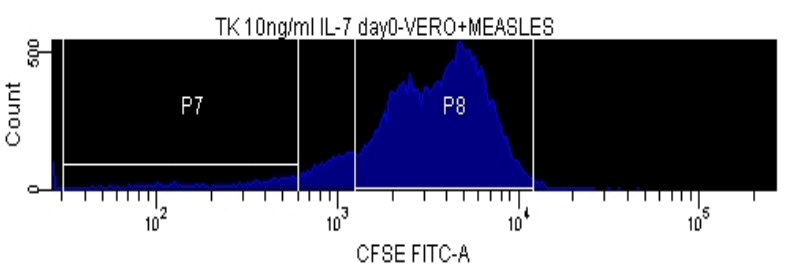
No Antigen



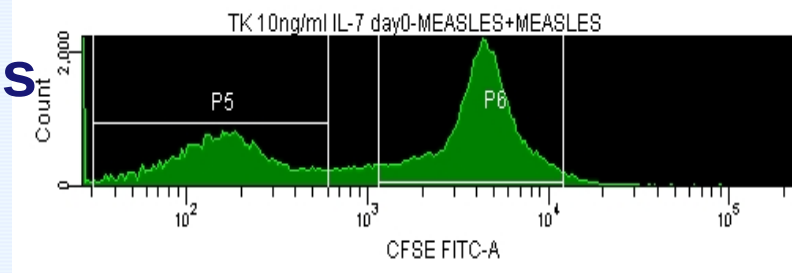


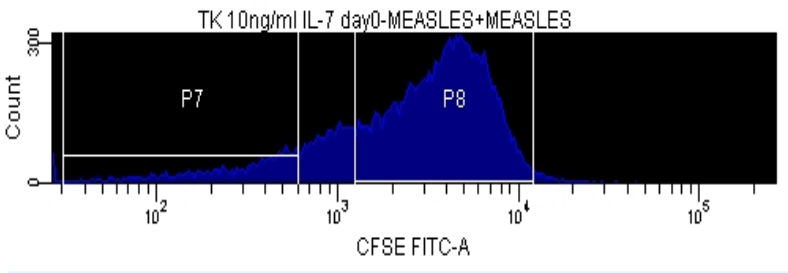
Vero





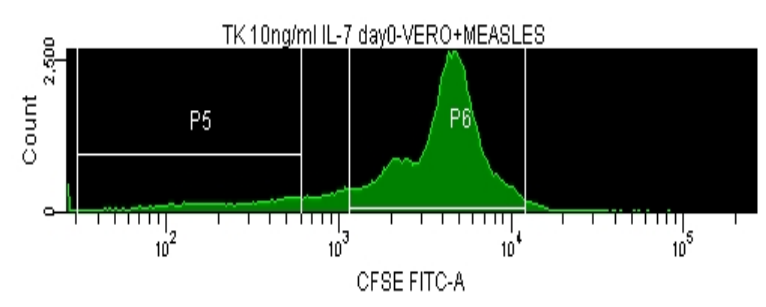
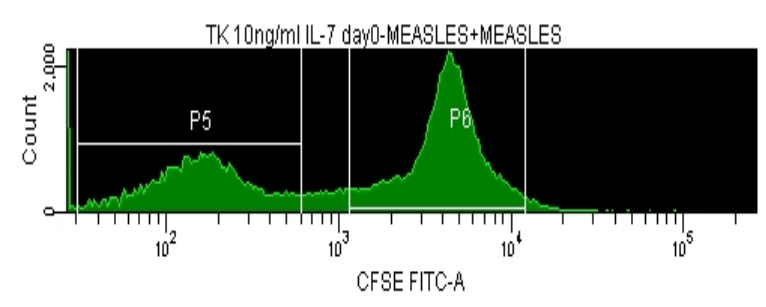
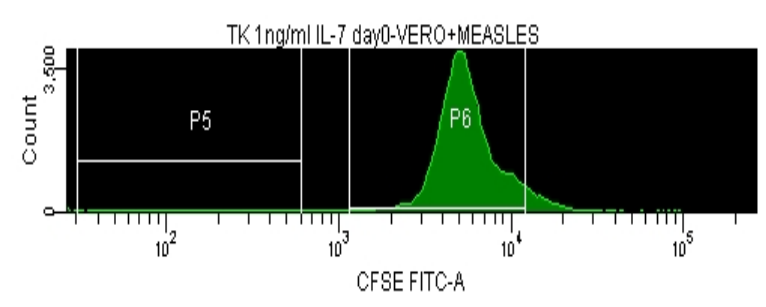
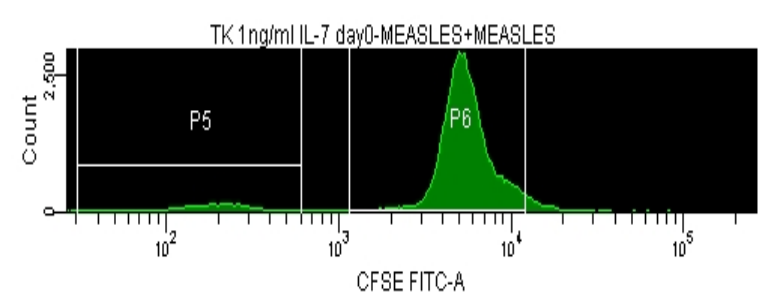
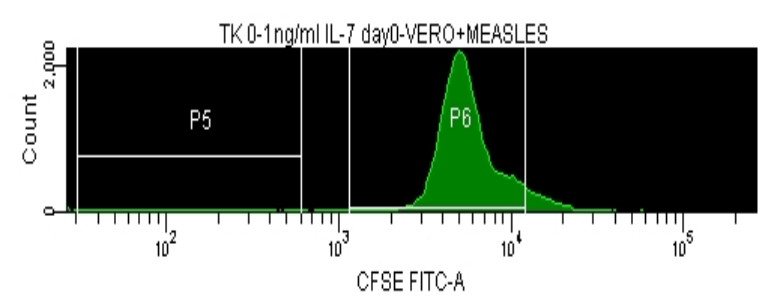
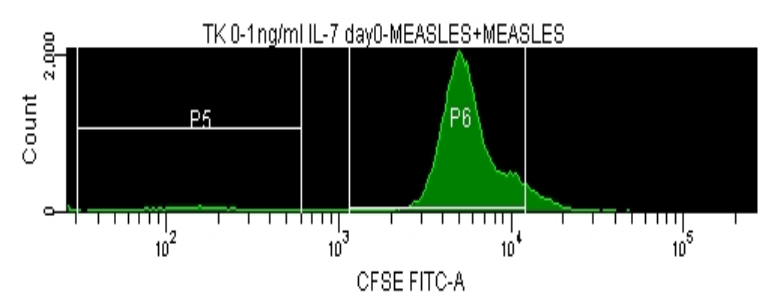
Measles



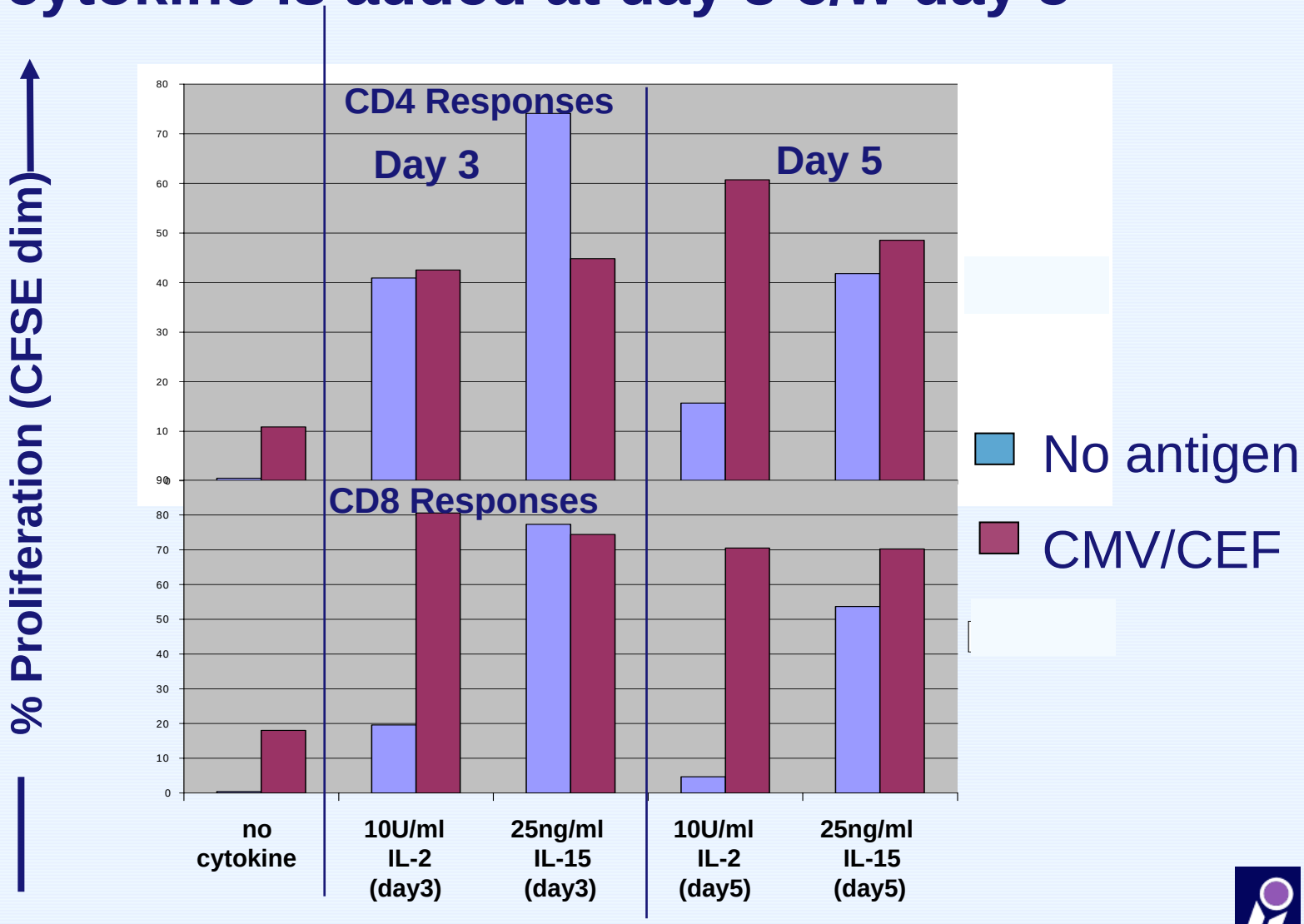


Non-specific background problematical

CD4 responses at varying concentrations of IL-7 added at Day 0

IL-7	Vero	Measles
10ng/ml		
1ng/ml		
0.1ng/ml		

Non-specific proliferation is increased when cytokine is added at day 3 c/w day 5



DNA prime/rFPV boost candidate vaccine trial

Immunisation dose and schedule

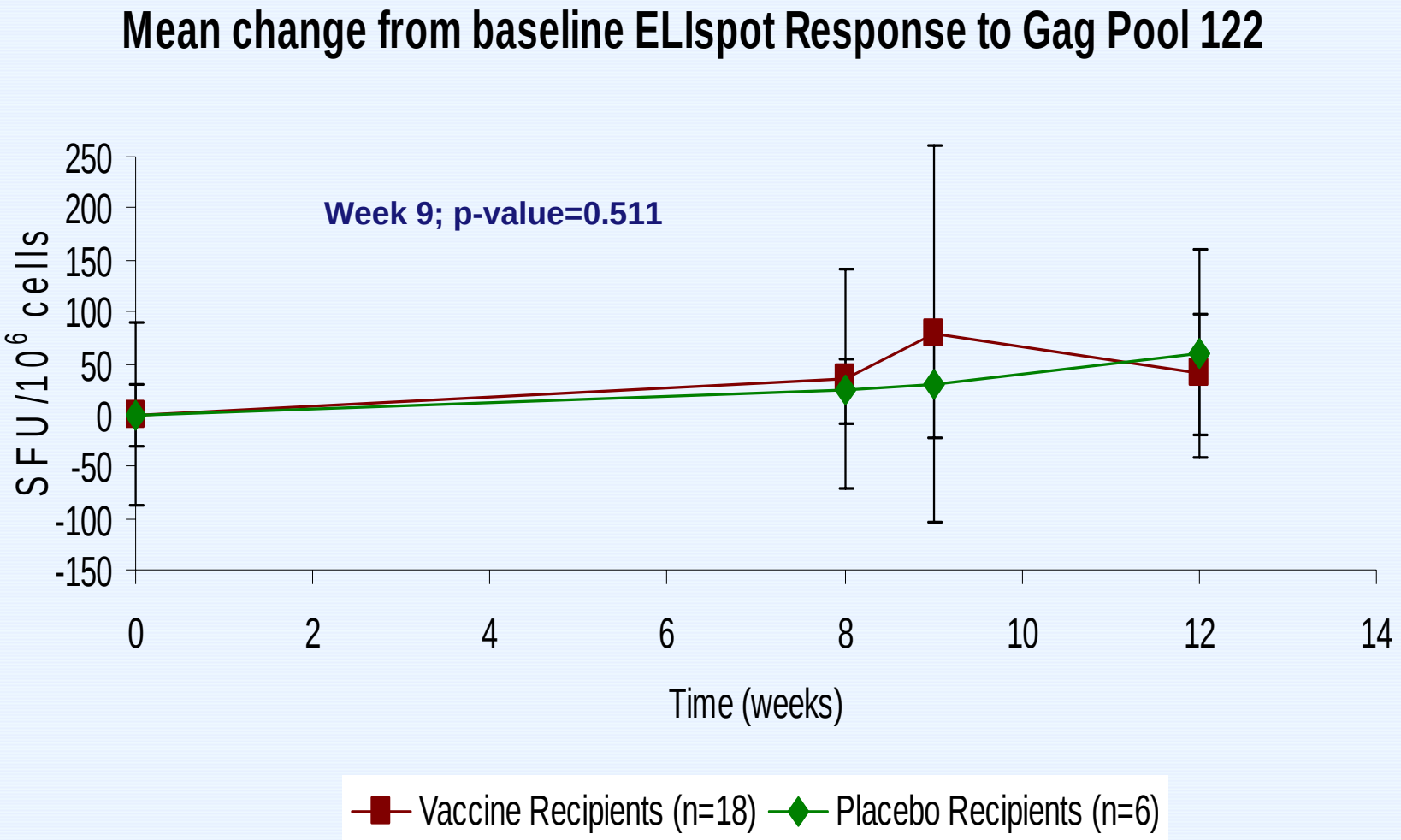
DNA; 1mg (IMI) on day 0 and week 4

rFPV; 5 x 10^7 pfu/mL (IMI) on week 8

Immunogenicity By ELISpot /ICC/Proliferation at wks 0, 8, 9, 12, 24

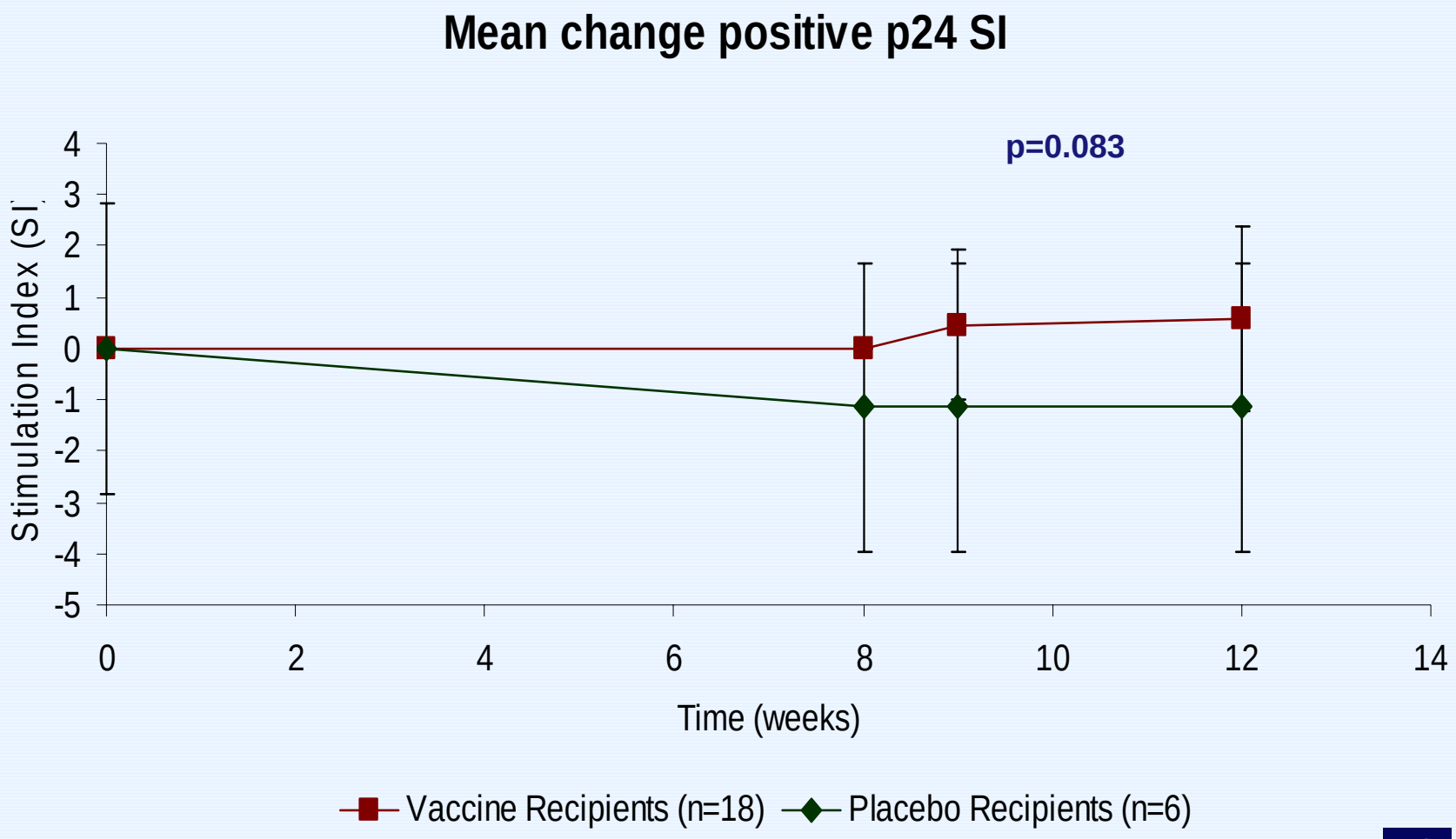
CAMPICC assay: conducted week 8 after 2 DNA vaccines

DNA prime/rFPV boost candidate vaccine trial

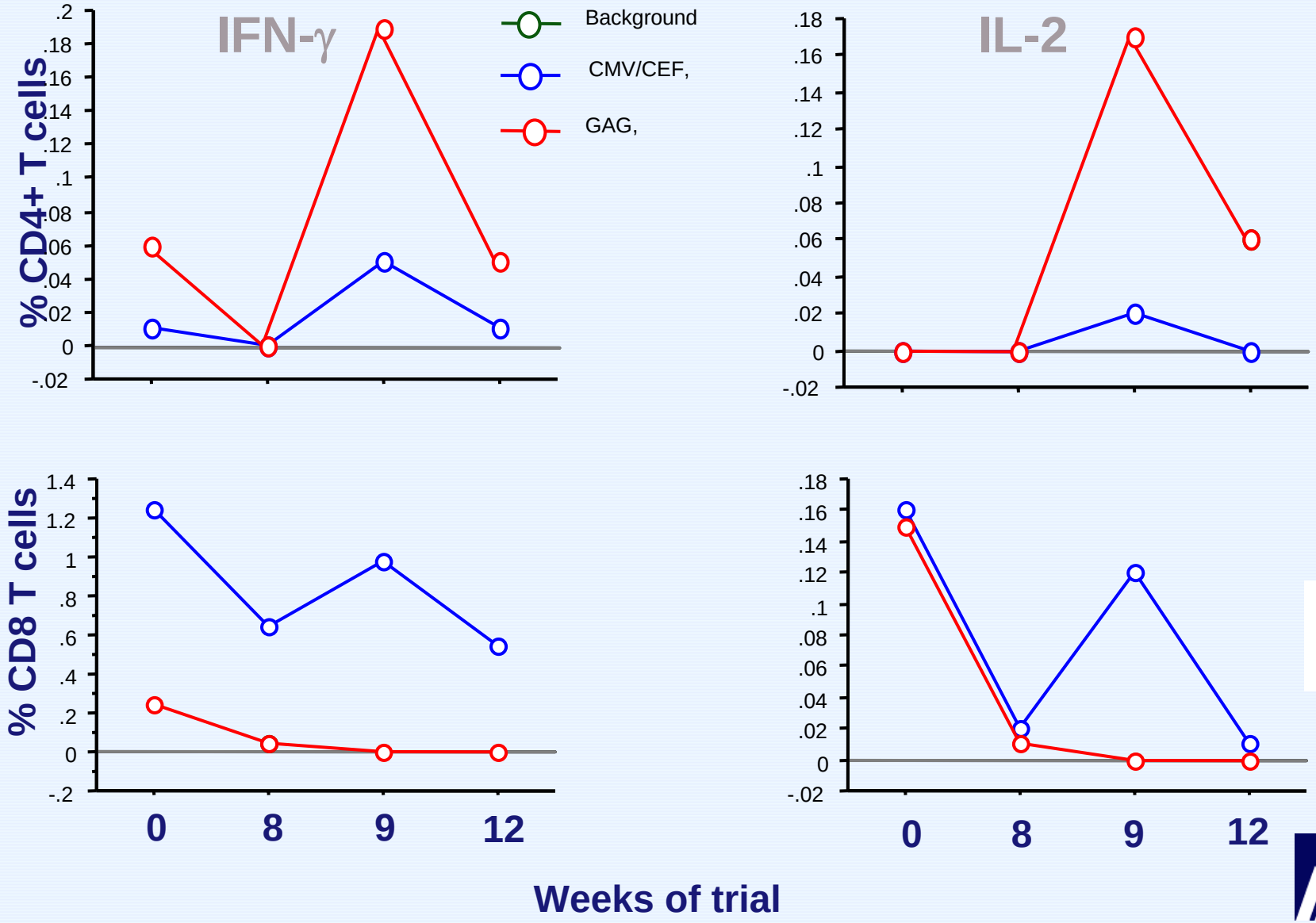


DNA prime/rFPV boost candidate vaccine trial

Lymphoproliferative responses

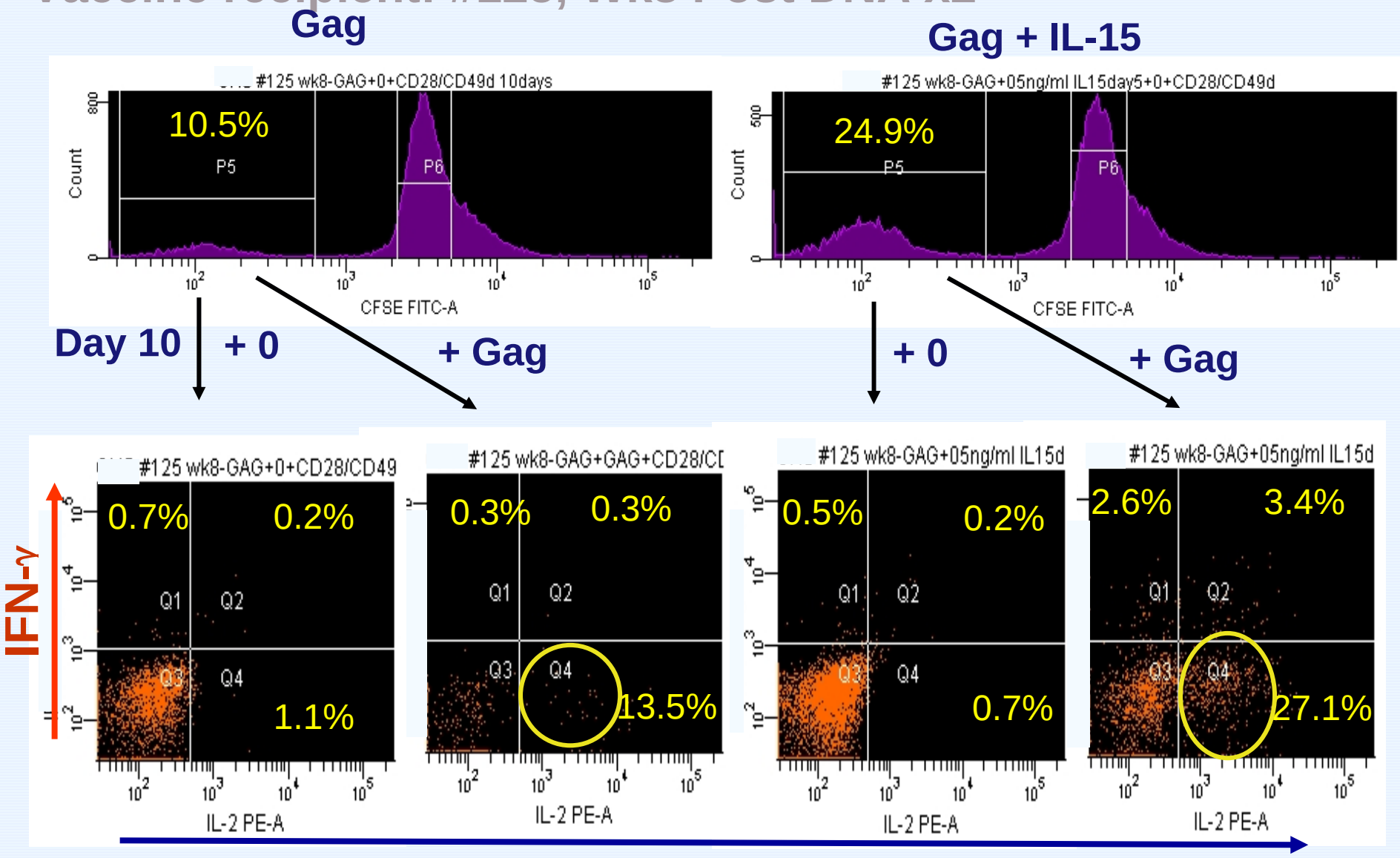


Vaccine responses appear to be CD4+ T cell mediated

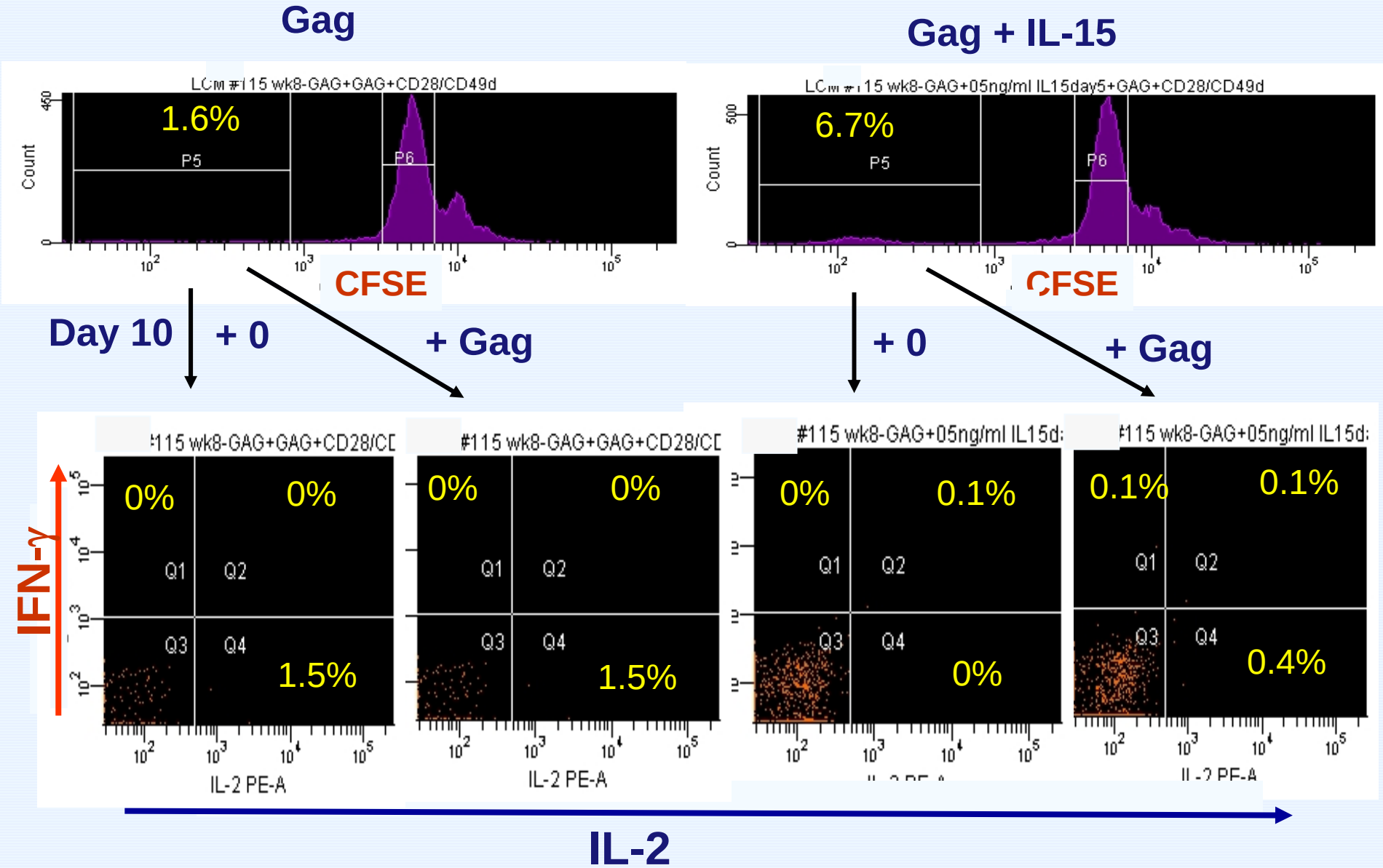


CAMPICC assay: CD4+ T cell responses:

Vaccine recipient: #125, Wk8 Post DNA x2



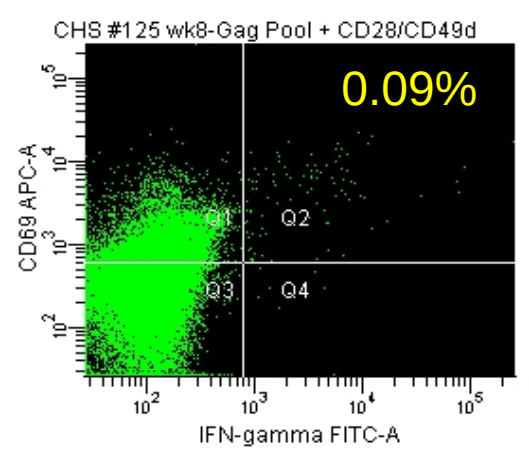
CAMPICC assay: CD4+ T cell responses:
Placebo recipient: #115, Wk8 Post DNA x2



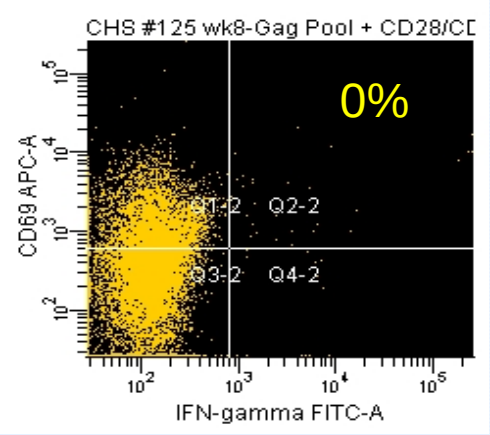
#125 - Vaccine Recipient - Responder
Week 8 (post DNA x2): ICC Results
Gag+(CD28+CD49d)

Background values subtracted

CD4

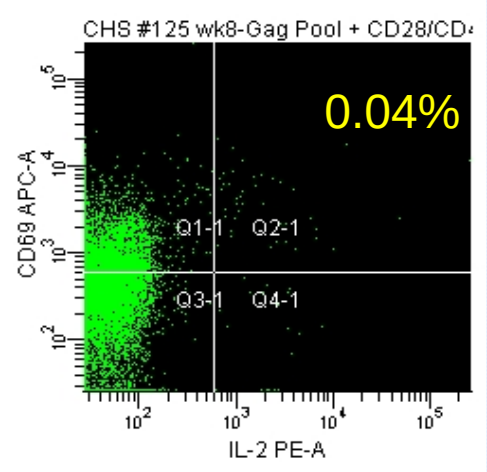


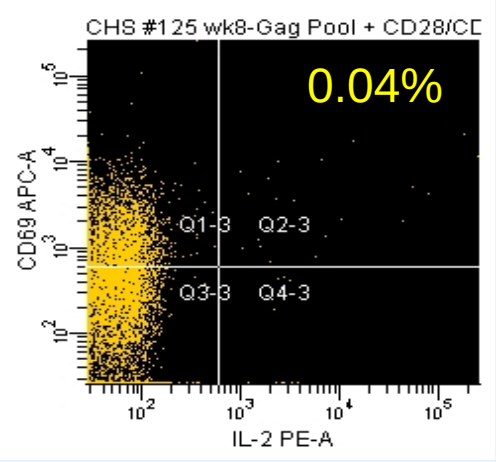
CD8



CD-69

IFN-gamma

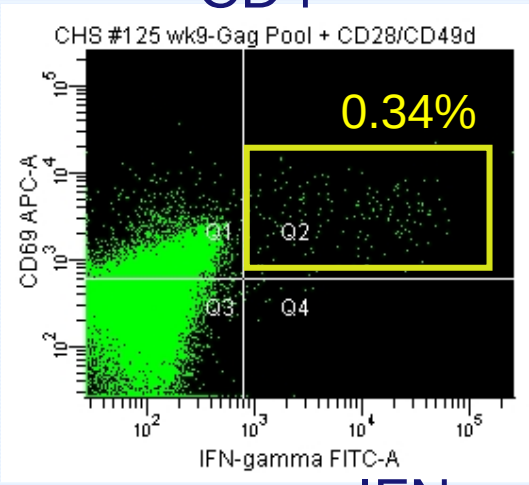




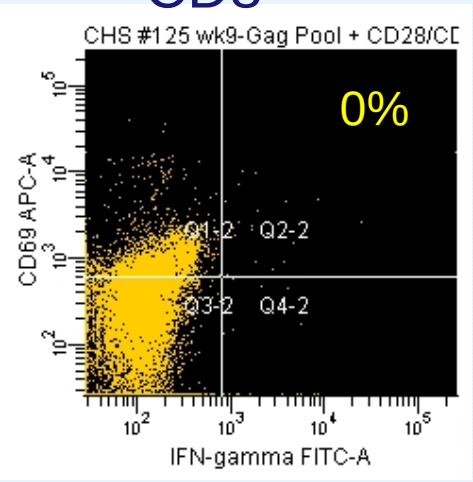
IL-2

#125 - Vaccine Recipient - Responder
Week 9 (Post FPV Boost): ICC Results
Gag+(CD28+CD49d)
Background values subtracted

CD4

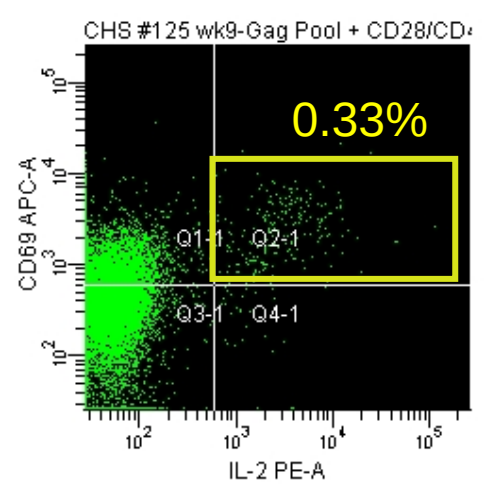


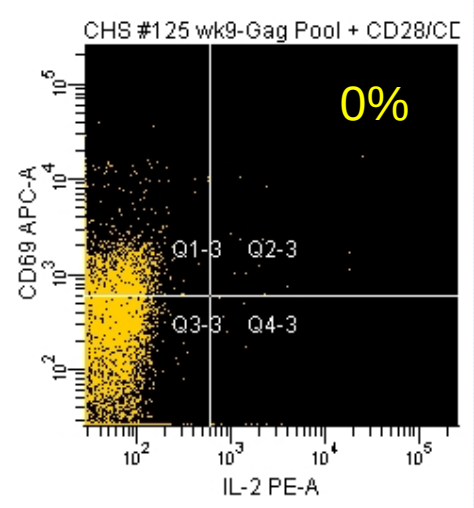
CD8



CD-69

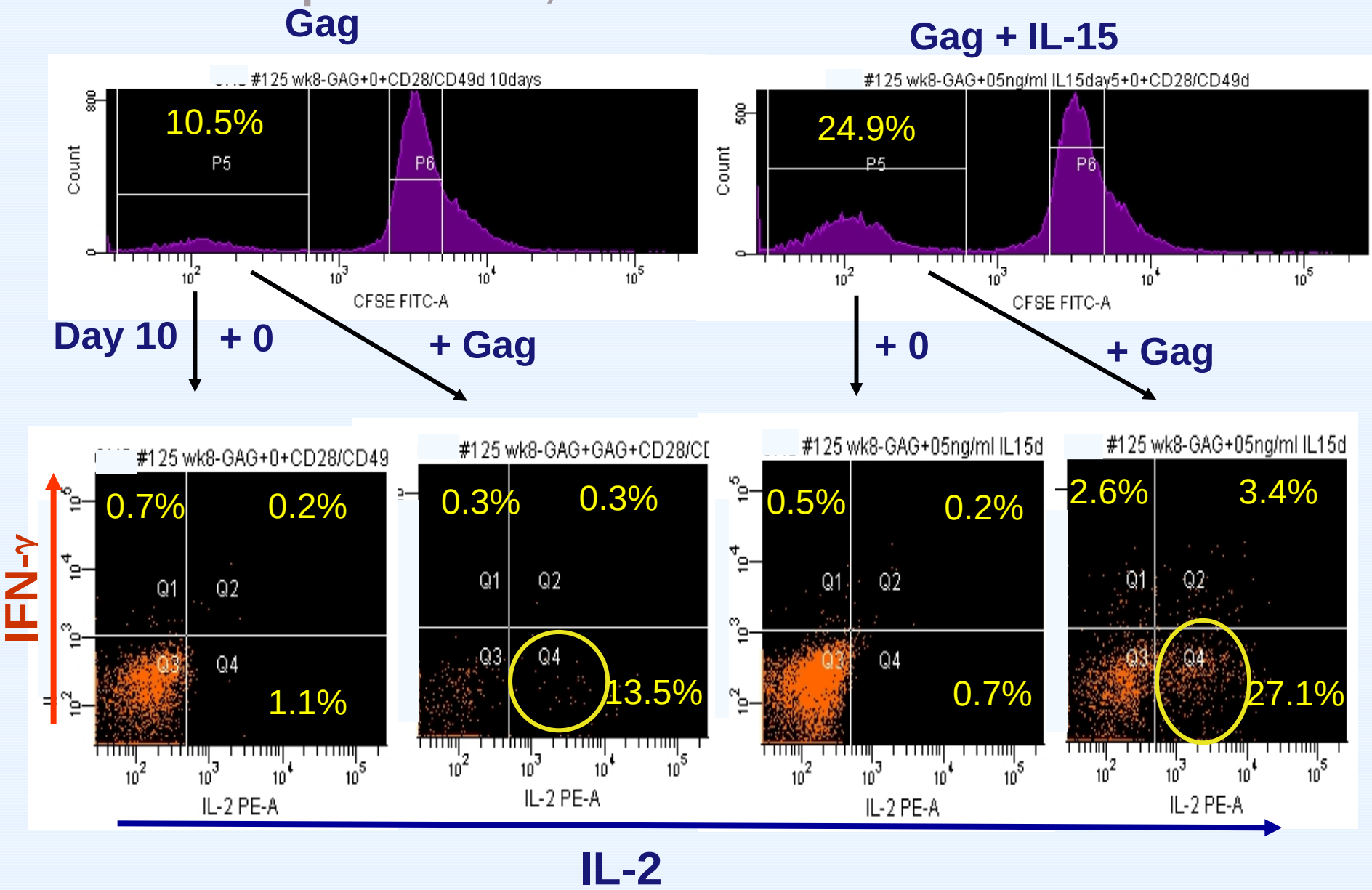
IFN-gamma





IL-2

CAMPICC assay: CD4+ T cell responses:
Vaccine recipient: #125, Wk8 Post DNA x2

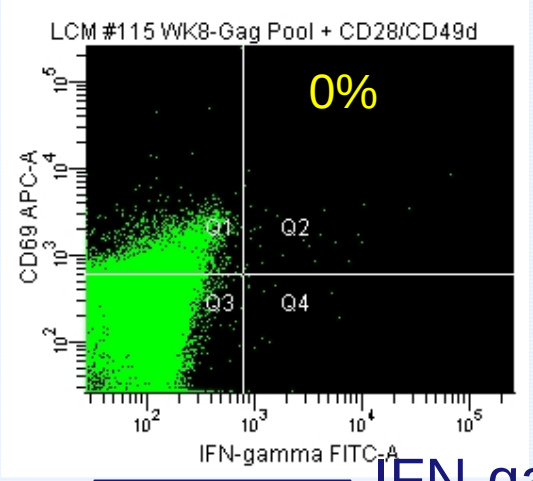


#115 - Vaccine Recipient - Placebo recipient Week 8(Post DNA x2): ICC Results

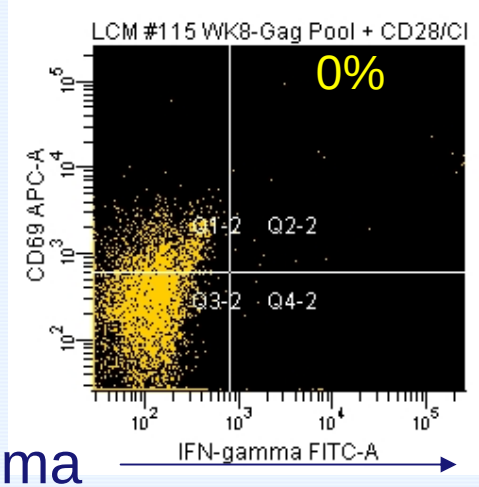
Gag+(CD28+CD49d)

Background values subtracted

CD4

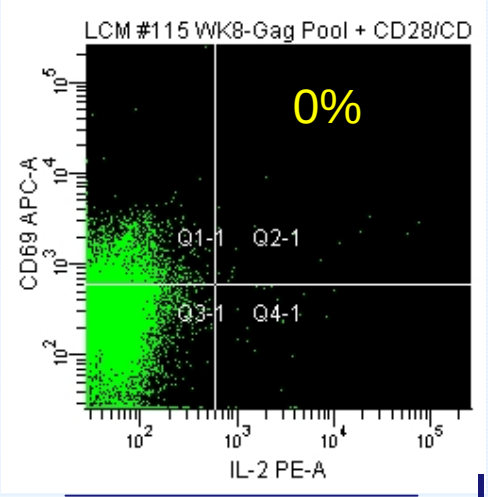


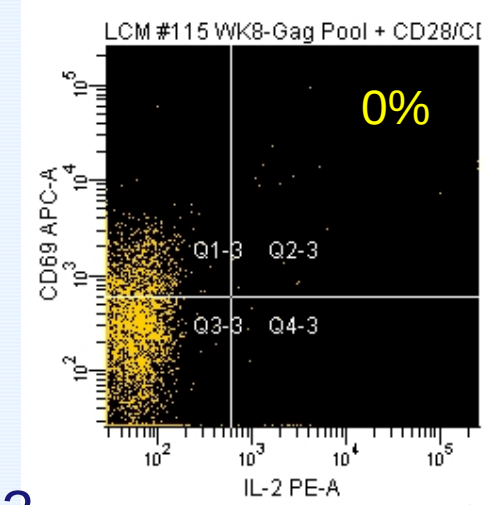
CD8



CD-69

IFN-gamma



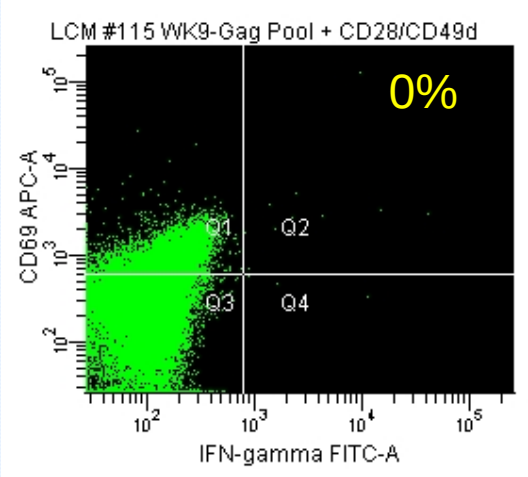


IL-2

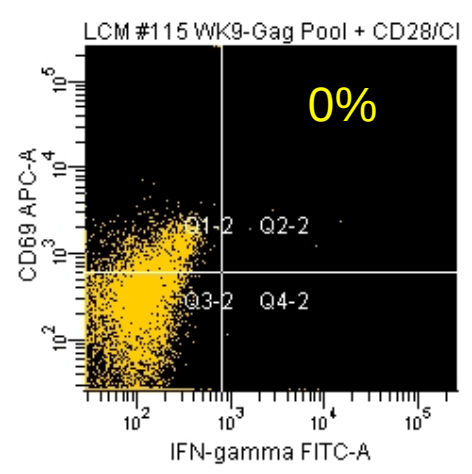
#115 - Vaccine Recipient - Placebo recipient
Week 9 (Post FPV Boost): ICC Results

Gag+(CD28+CD49d)

CD4

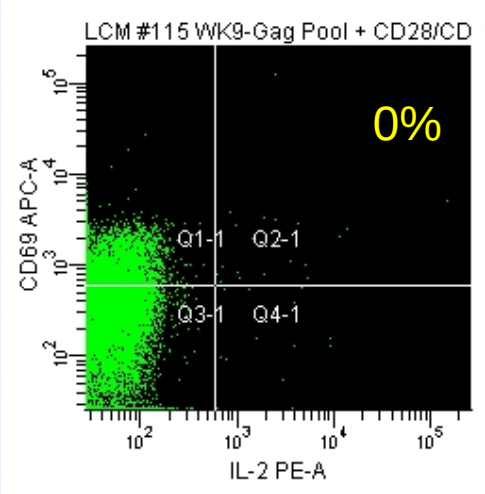


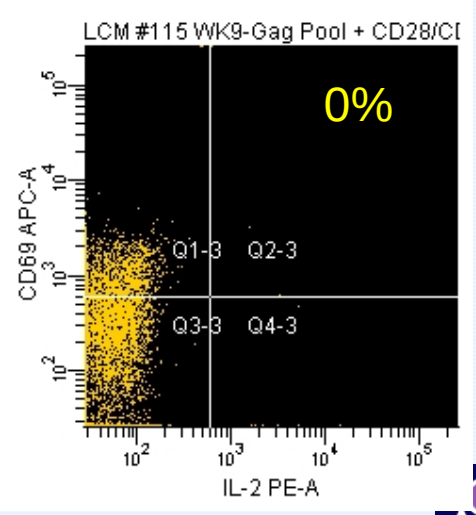
CD8



CD-69

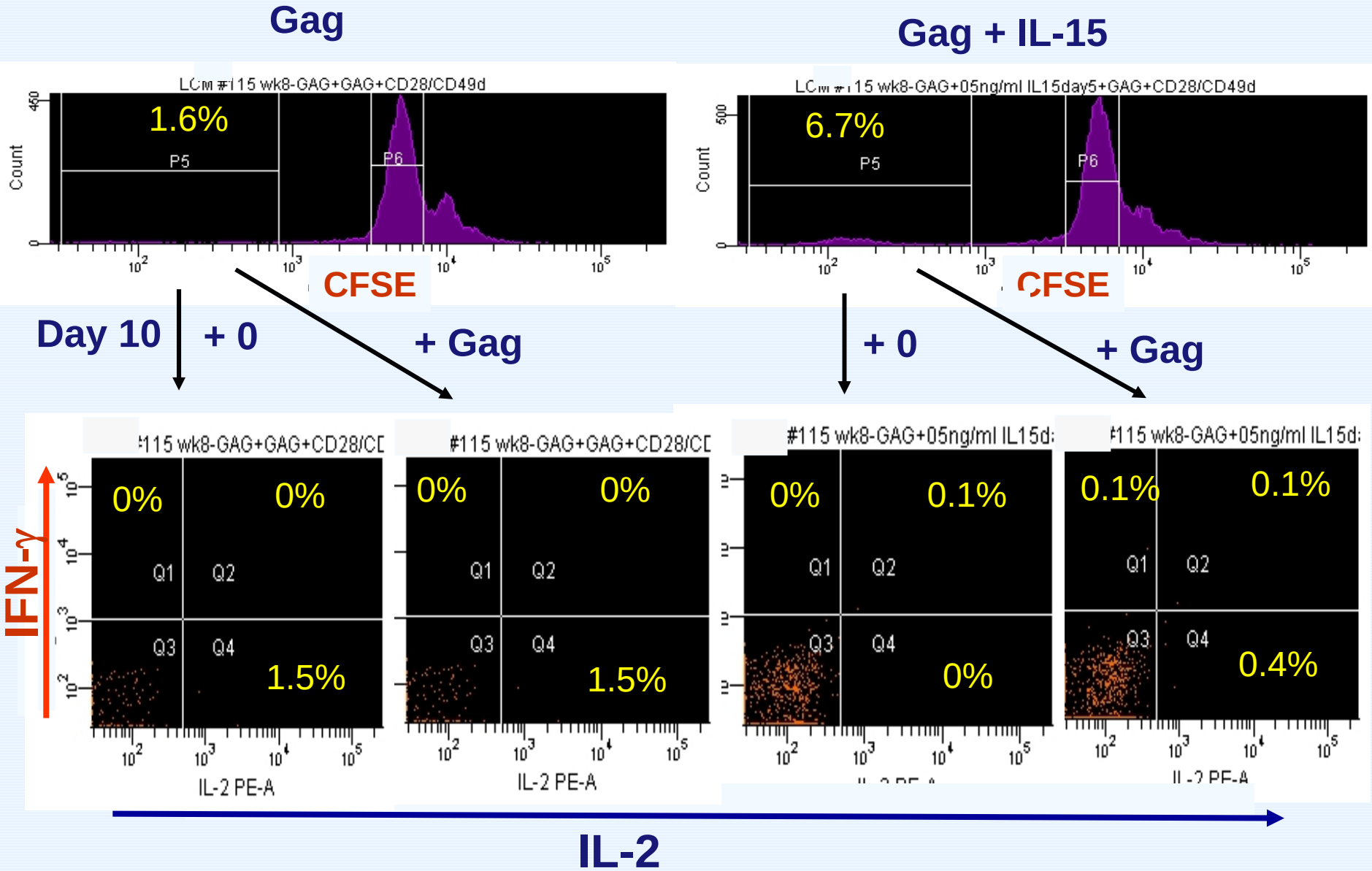
IFN-gamma





IL-2

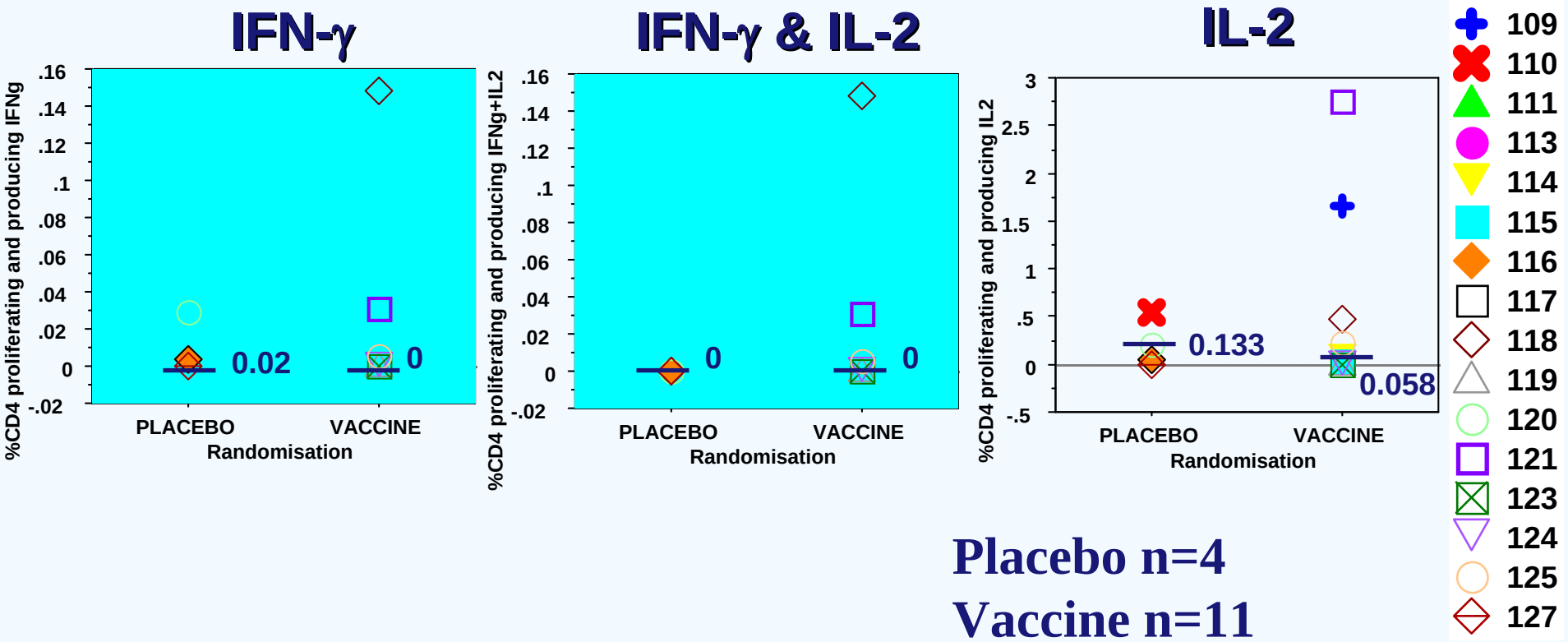
CAMPICC assay: CD4+ T cell responses:
Placebo recipient: #115, Wk8 Post DNA x2



VACCINE vs PLACEBO

GAG culture re-challenged with GAG

% CD4 proliferating and producing…



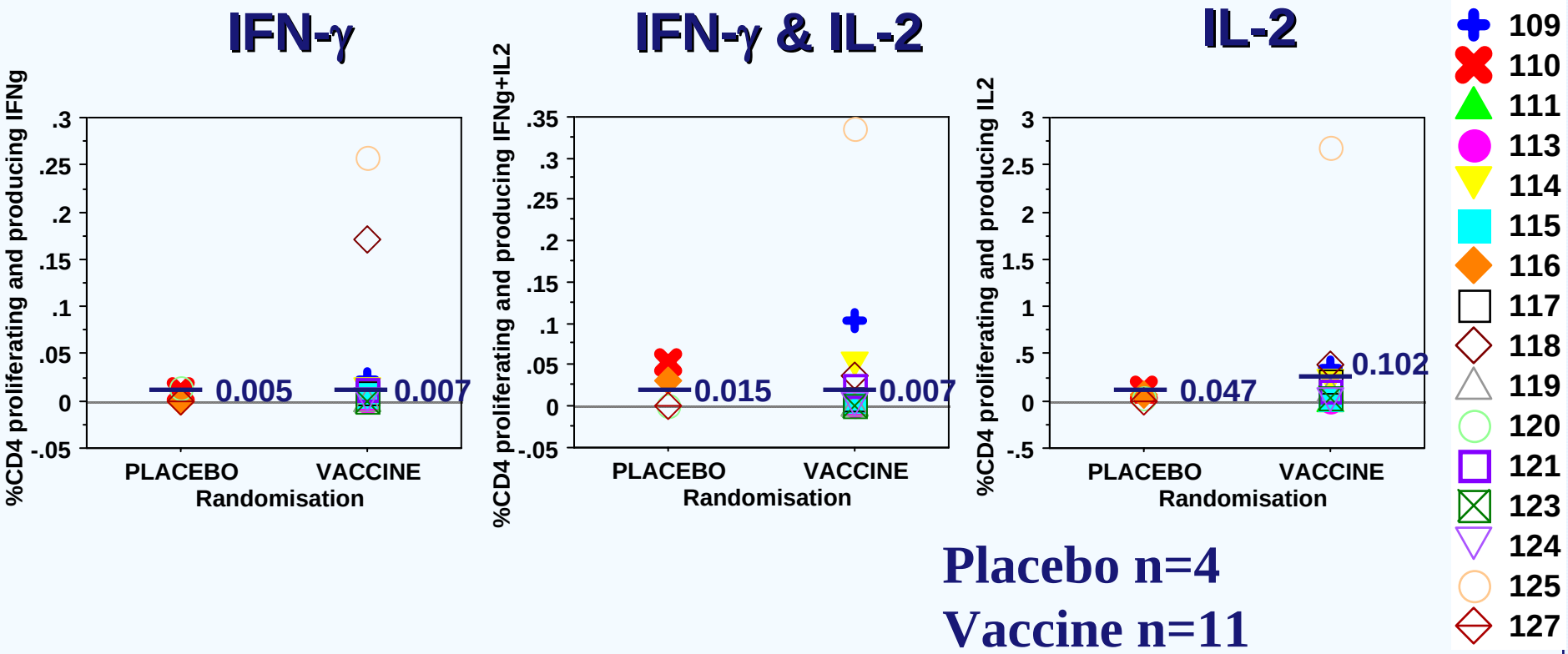
Placebo n=4

Vaccine n=11

VACCINE vs PLACEBO

GAG culture + IL-15 re-challenged with GAG

% CD4 proliferating and producing…



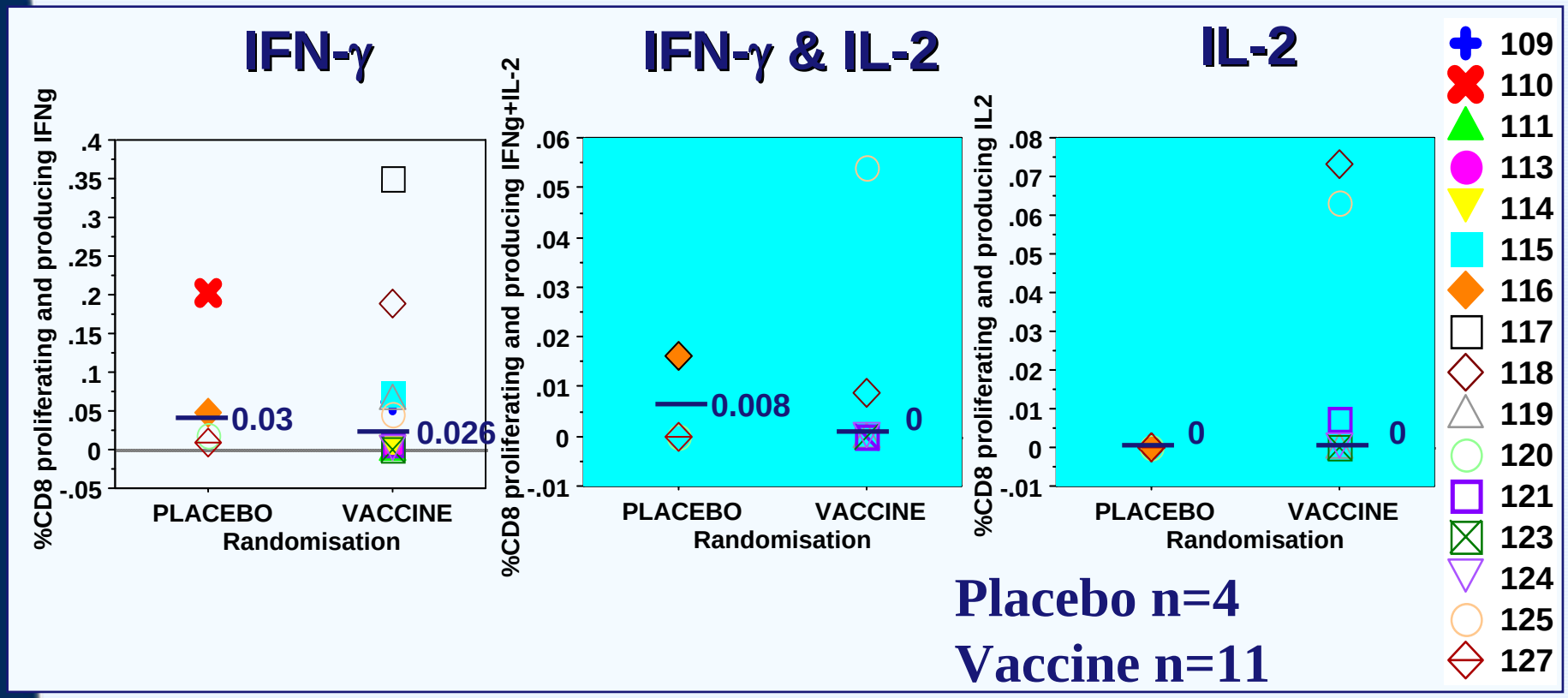
Placebo n=4

Vaccine n=11

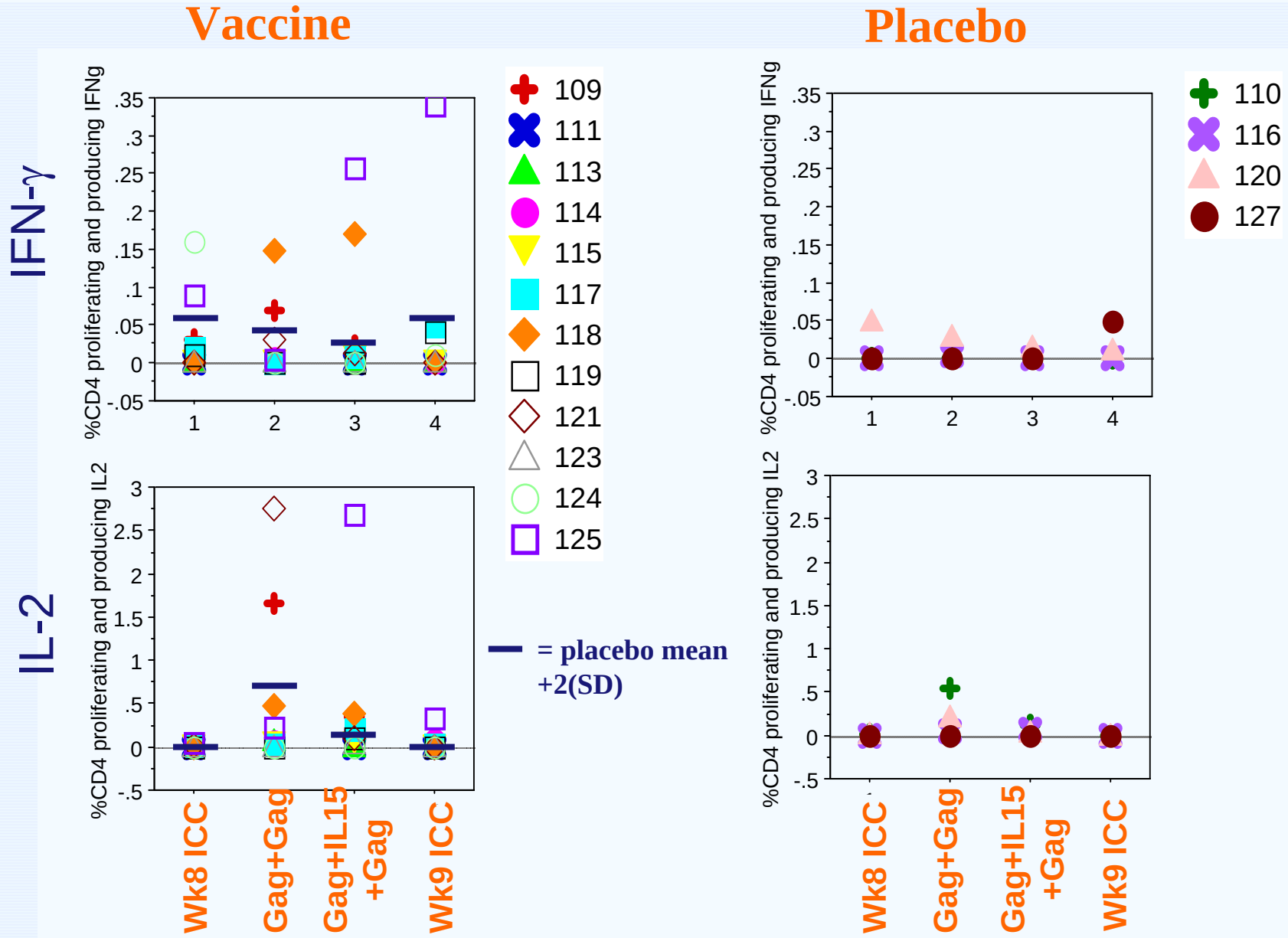
VACCINE vs PLACEBO

GAG culture + IL-15 re-challenged with GAG

% CD8 proliferating and producing…



CD4 Responses
Week 8 & 9 ICC results vs CAMPICC assay



CAMPICC assay: Results

Performed on last 16 individuals recruited

- Evaluable data on 15: 11 active, 4 placebo
- 4 recipients of active vaccine had either IL-2 or IFN-γ production from CD4 that was > mean +2 SD of the placebo responses,
- 1 recipient had CD8 response that fulfilled the same criteria

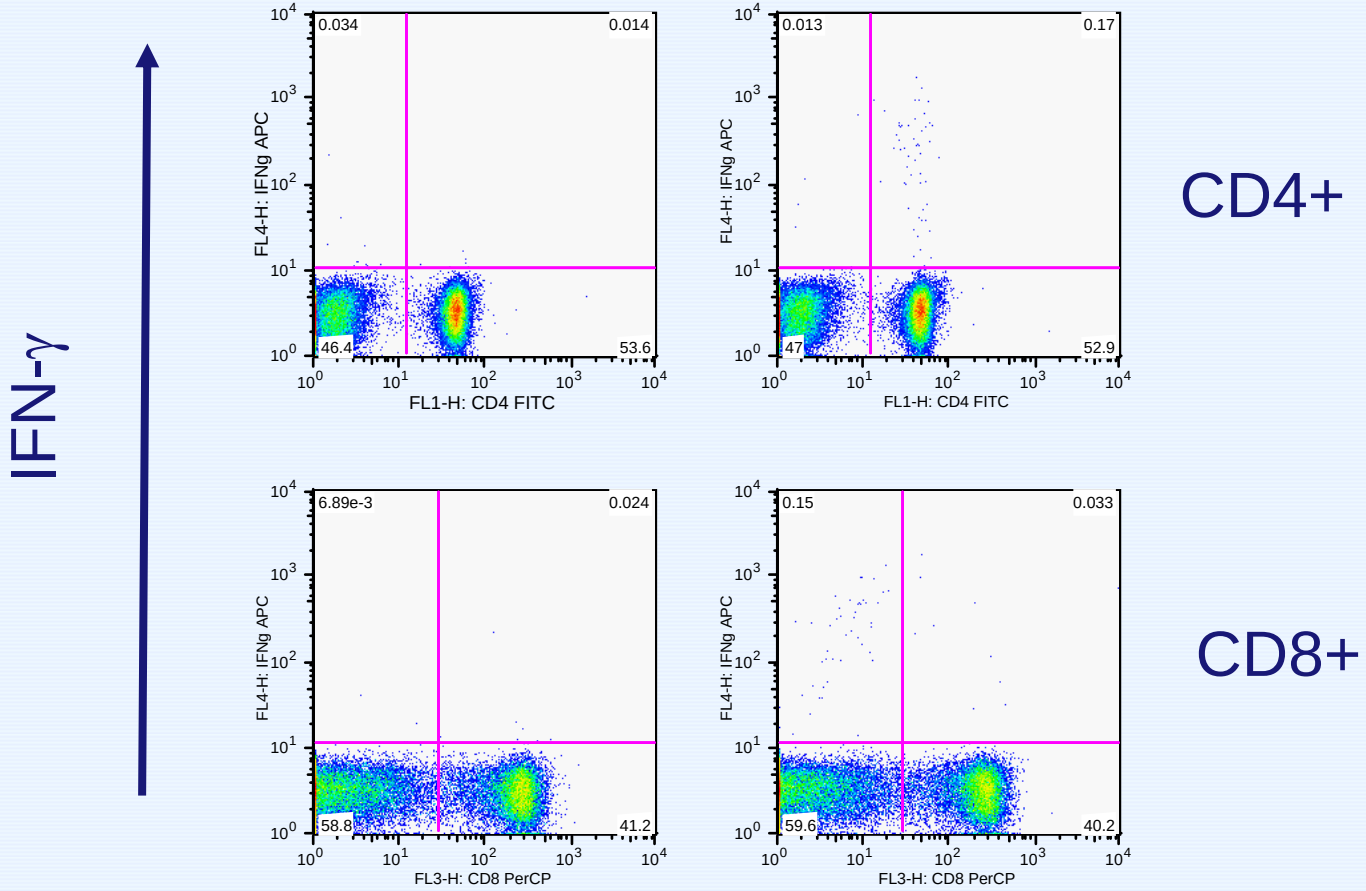
DNA may be priming 1/3 of the recipients

Not all of these boosted with rFPV

Suggests both components sub-optimal

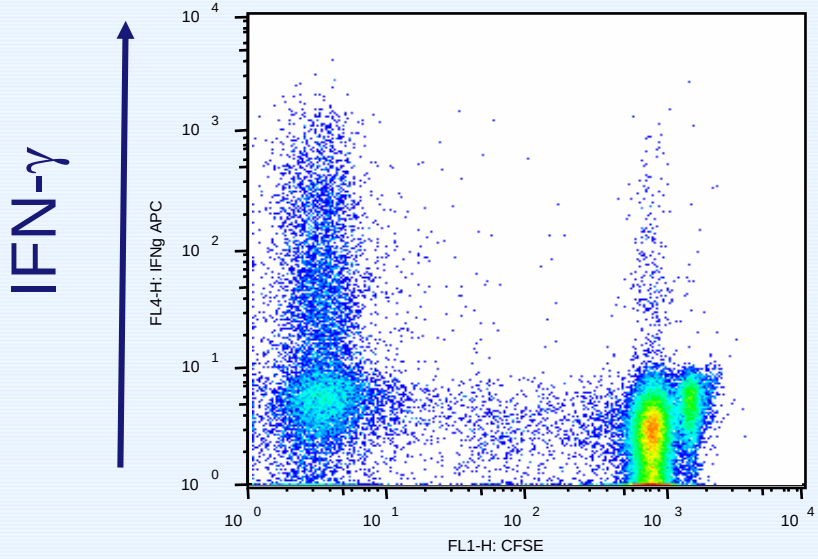
Confirm by conducting optimised assay on all 24

ICS after two DNA vaccines: Macaques

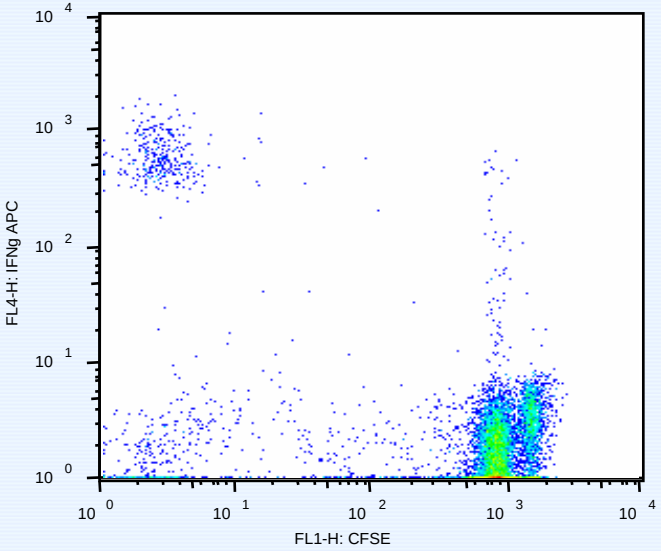


ICS following 10 day incubation with CFSE and IL7 (1ng/mL): macaque

CD3+ CD4+ IFNγ+
Proliferating cells



CD3+ CD8+ IFNγ+
Proliferating cells



Comments/Conclusions

Assay initially worked up on robust responses:
? Appropriate methodology for borderline responses?

Unable to be validated in trial already conducted because of ineffective *in vivo* boost

Responses dominated by CD4, surprising, esp since using IL-15 (CD8 supporting cytokine) but is consistent with ICC data and data from similar vaccine approaches

IL-15 most effective cytokine so far in humans ? in macaques

Exploring other cytokines and cytokine combinations with Ag that induce less robust responses

Repeat analysis with fully optimized assay on cryo-preserved cells from this trial and proposed high dose phase I trial

Eliminate peptides driving “non-specific”/ cross reactive responses

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