

# Human DC vaccination with HIV antigens

Mary Marovich, MD, DTM&H

Division of Retrovirology, WRAIR

USMHRP

Uniformed Services University Health Sciences

AIDS Vaccine Meeting, Seattle, WA

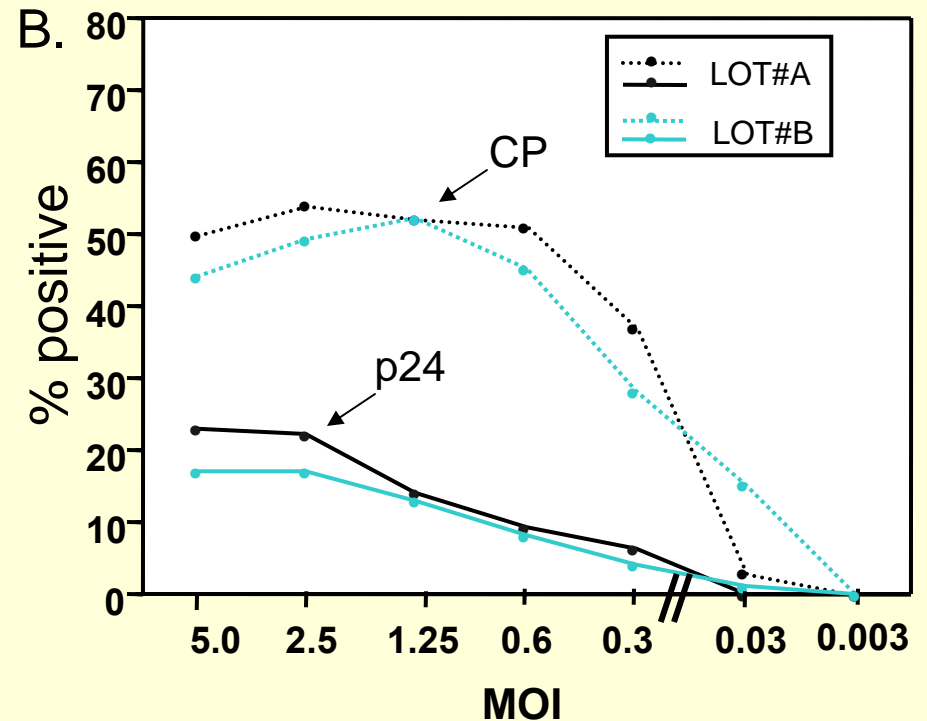
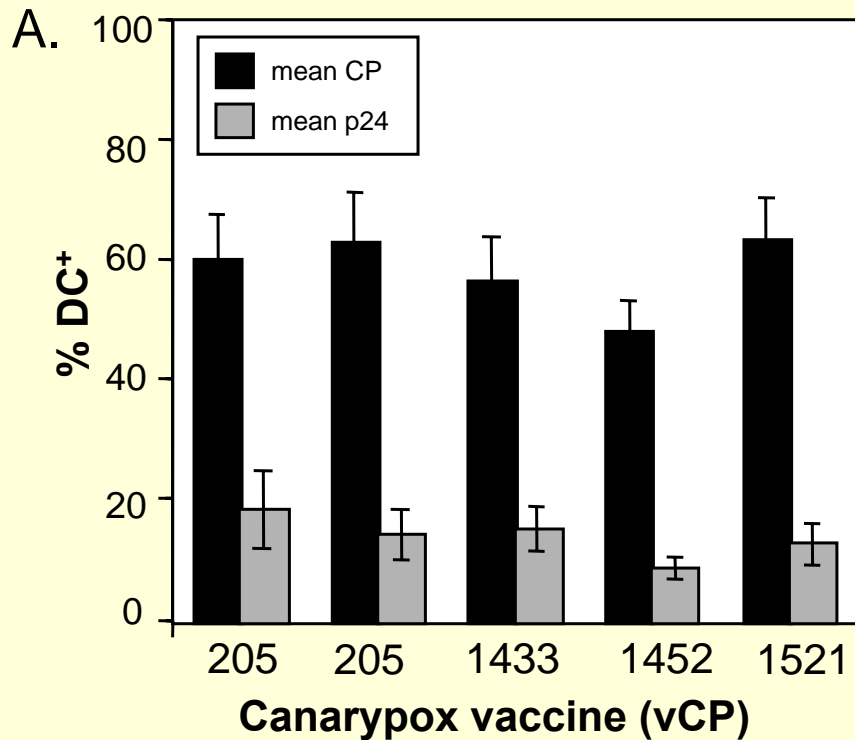
August 22, 2007



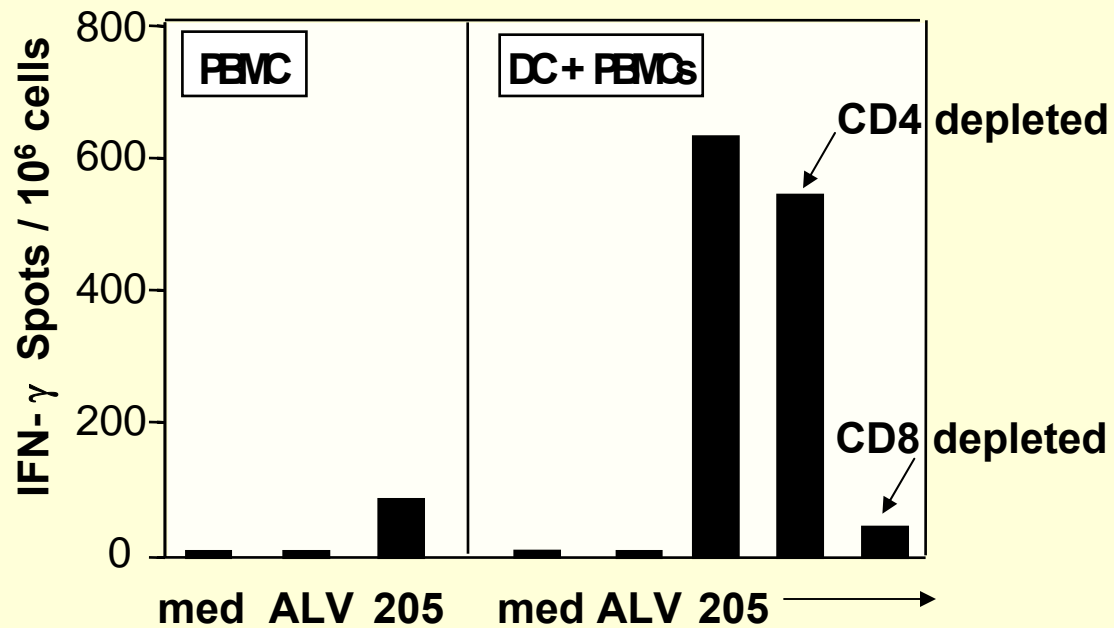
# New vaccine strategy to directly target DC

- Using cGMP produced, safe pox based HIV vaccine
- Enhance HIV Ag presentation and optimize Ag delivery

## Similar infection and gene expression in DC with ALVAC vaccines

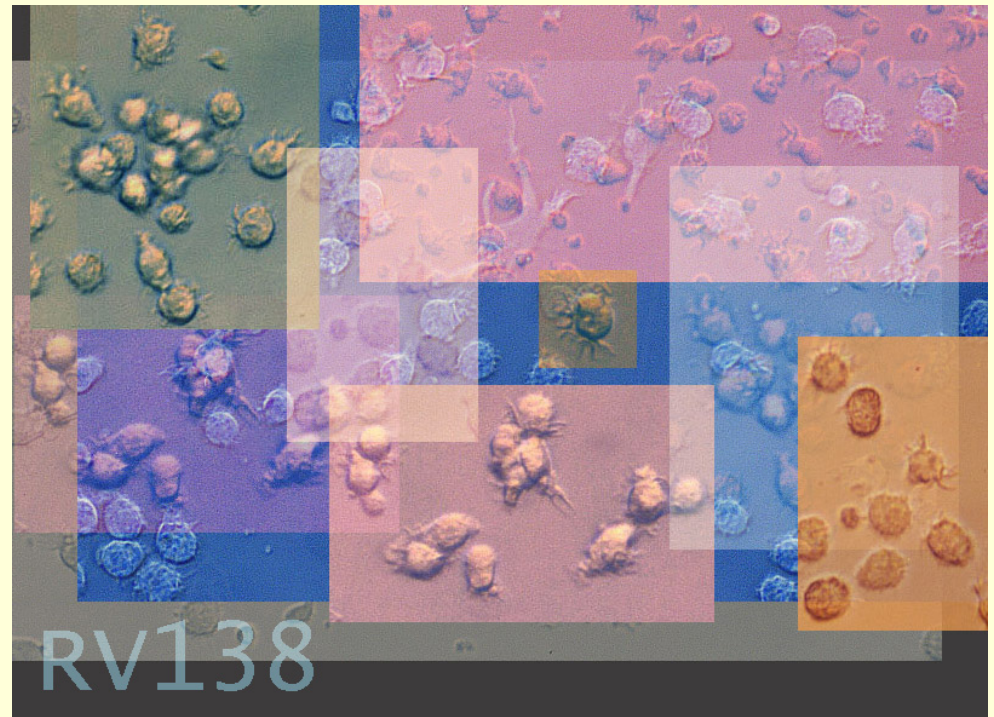


# vCP205-DC reactivate autologous primed Ag specific CD8



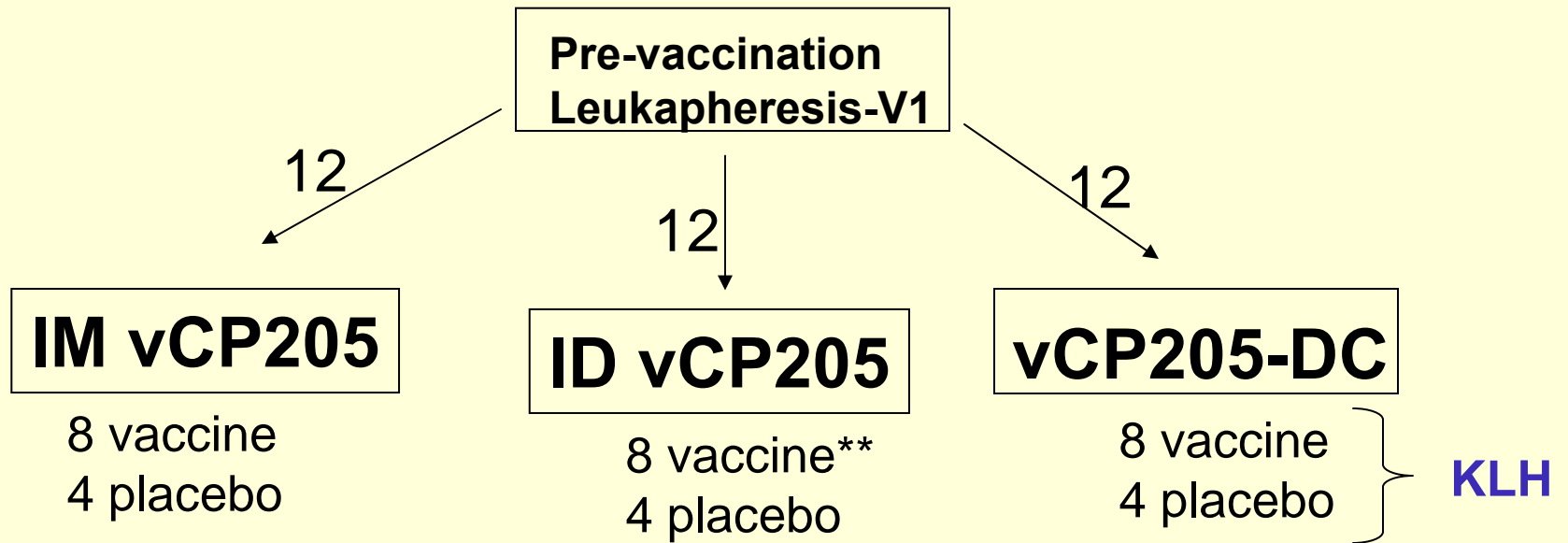
## RV138 Overview

- Clinical trial with some unique features
- Preventative vaccine strategy in healthy individuals
- Autologous DC vaccine for an infectious disease (typically DC only used in cancer patients)
- Direct comparison of DC-delivered vaccine vs conventional vaccine delivery routes (ID and IM)
- Leukapheresis pre/post vaccination allowed extensive study of immune response



# RV138 Study Design

(n = 36) Seronegative Volunteers



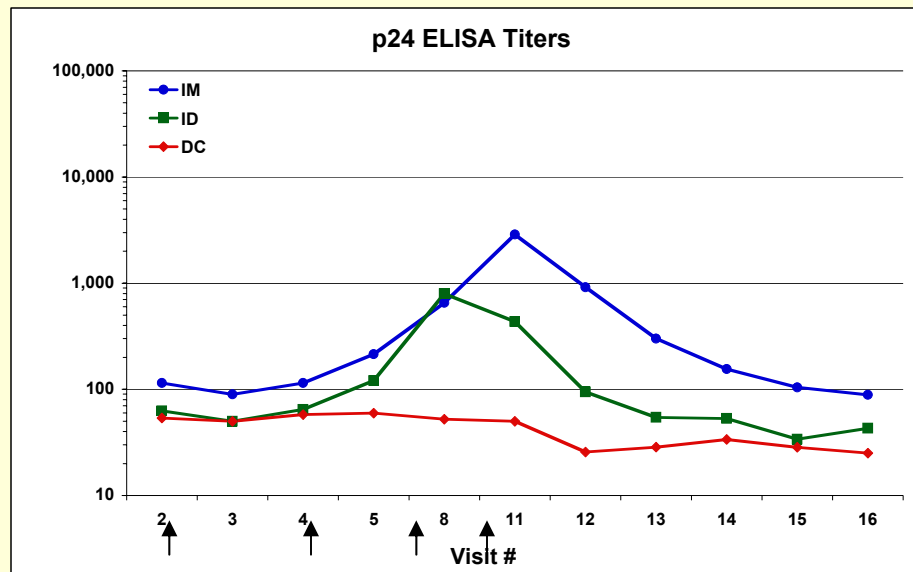
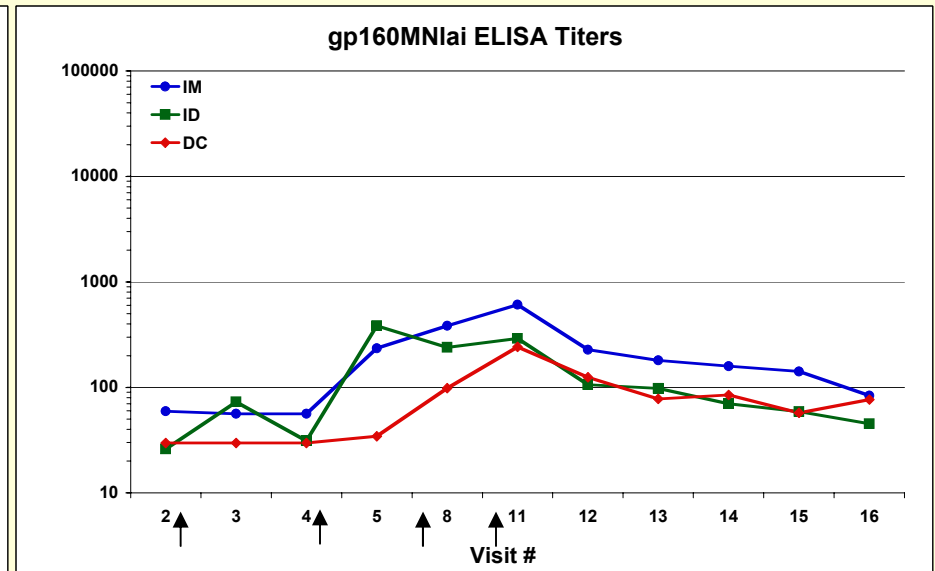
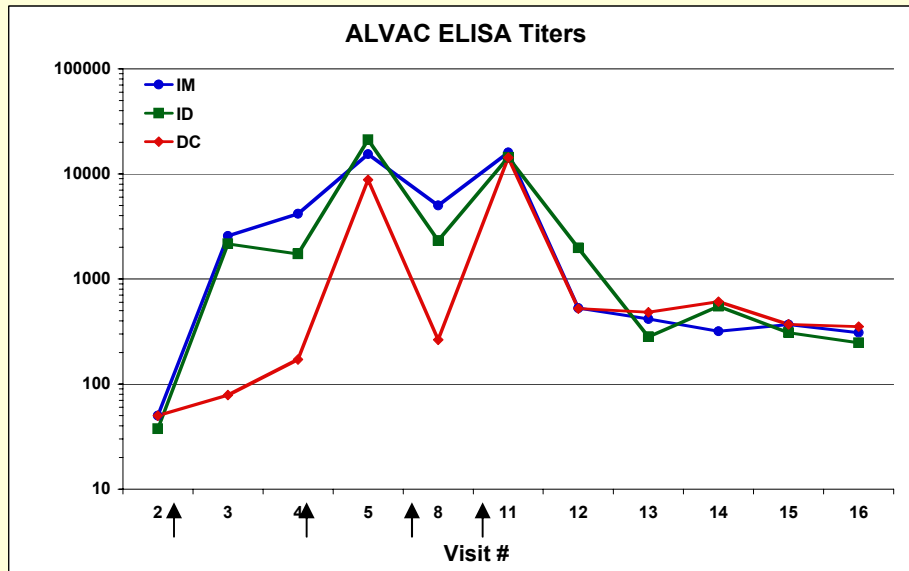
Vaccinate 0,1,3 and 6 mos

Post-vaccination  
Leukapheresis-V11

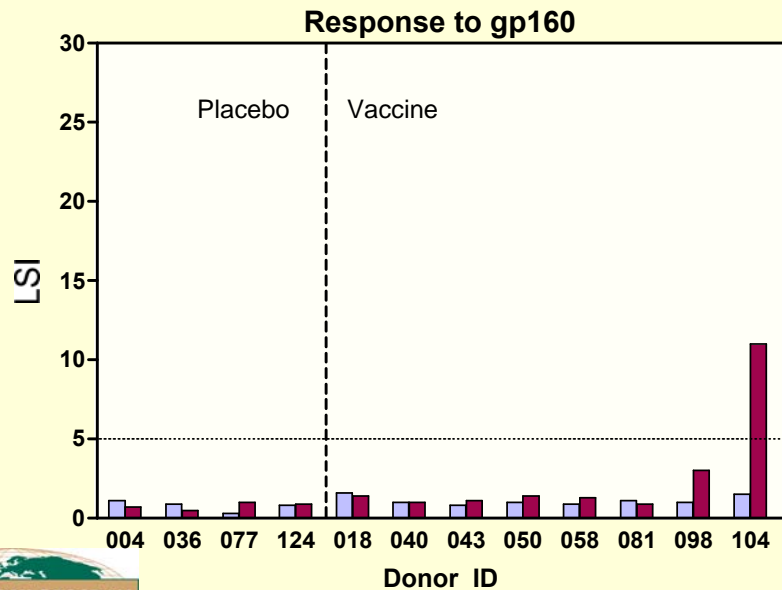
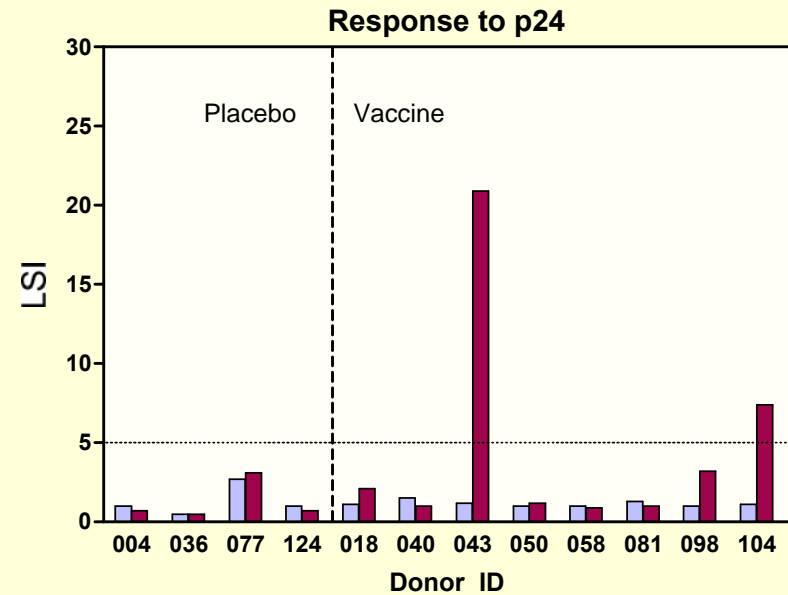
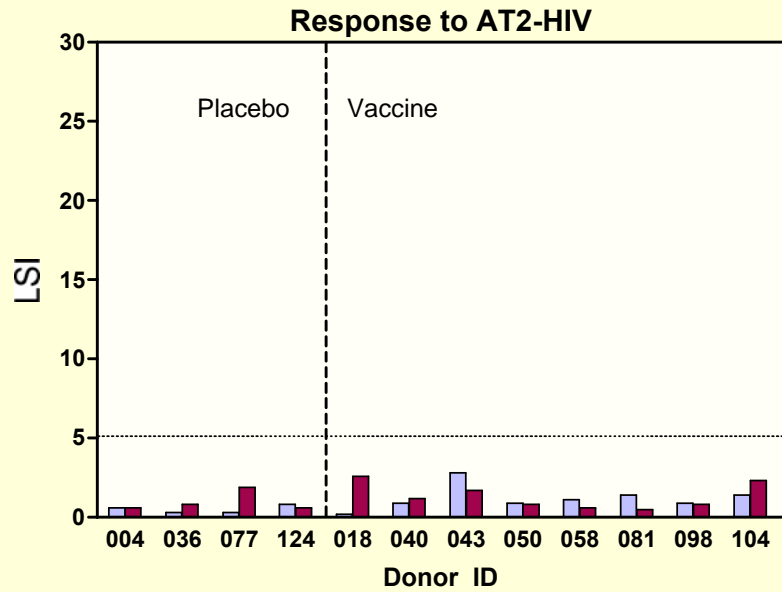
\*\* 50% dose



# Different kinetics and potency of Ab responses in DC group



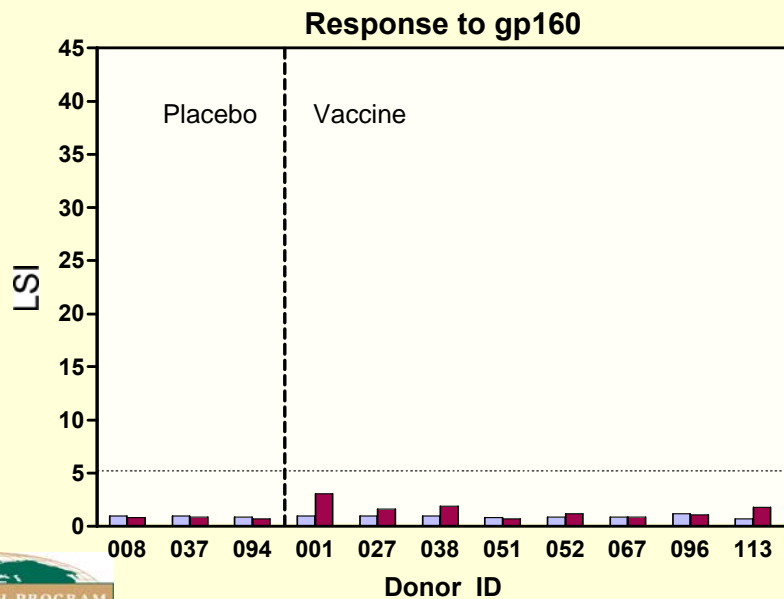
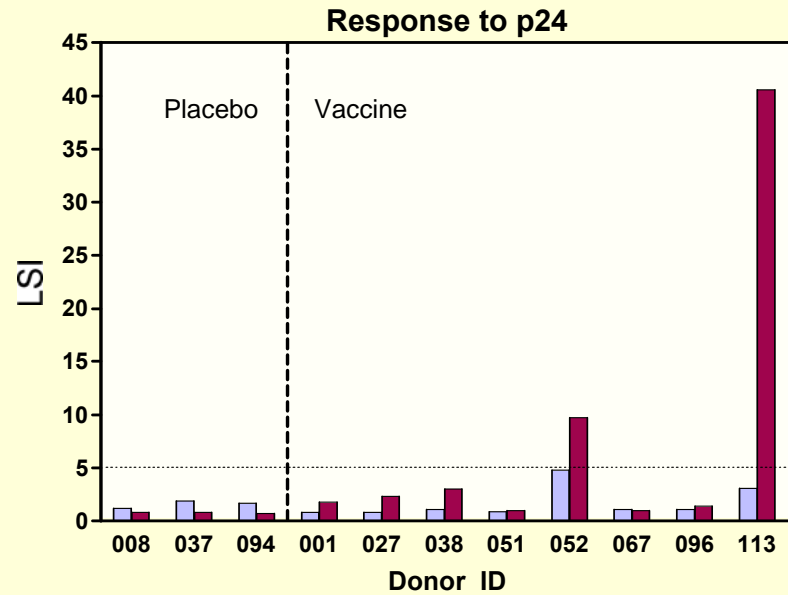
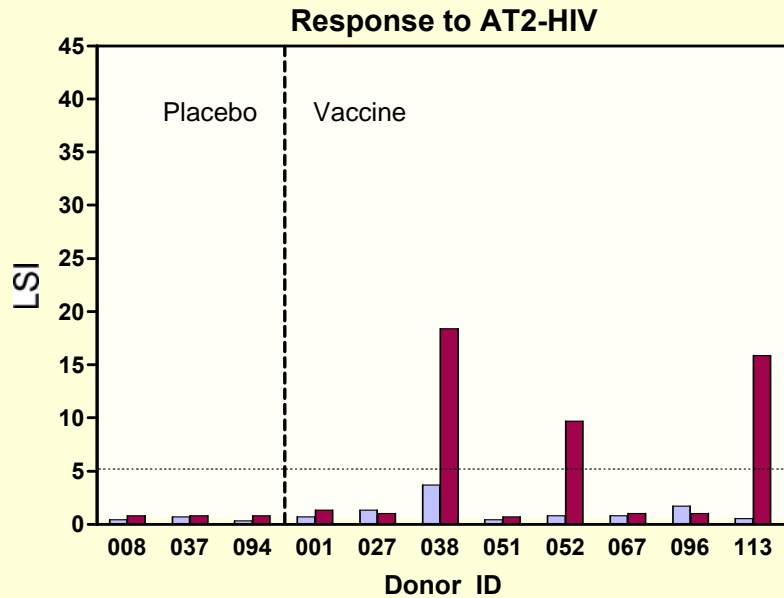
# Limited pattern of LPA responses in ID arm



Pre-vaccination  
 2 weeks post-final vaccination



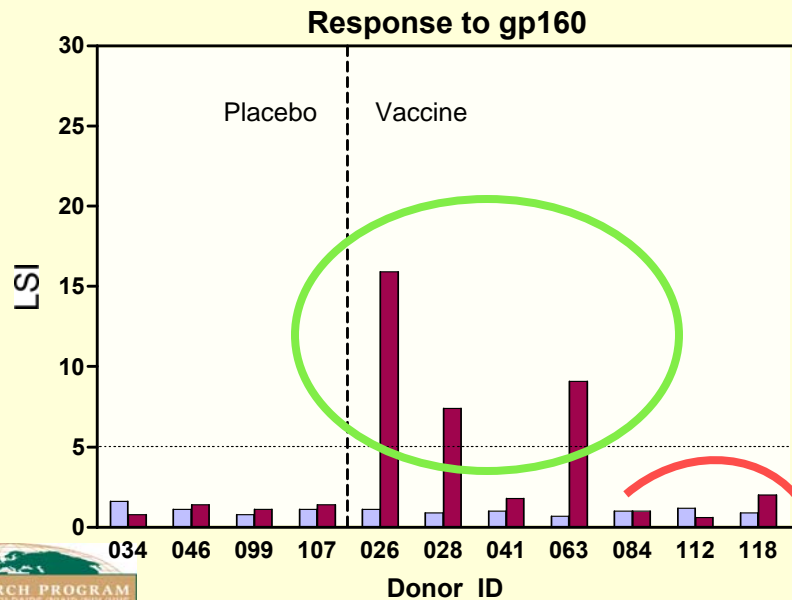
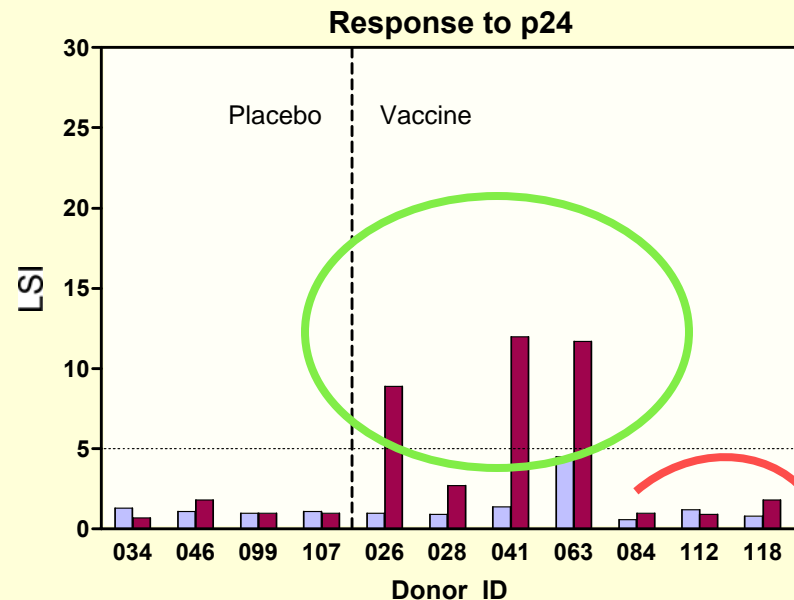
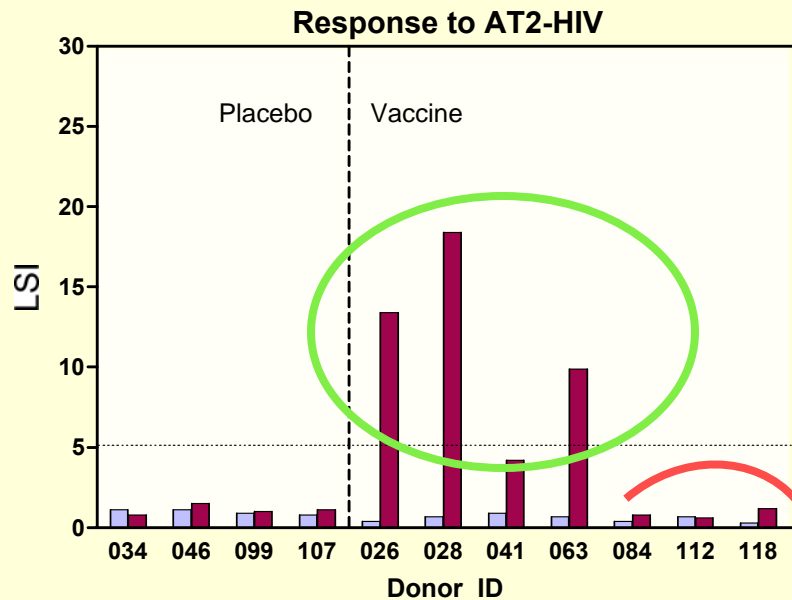
# Limited pattern of LPA responses in IM group



Pre-vaccination  
 2 weeks post-final vaccination



# Increased breadth of HIV Ag specific responses in DC arm



Pre-vaccination  
 2 weeks post-final vaccination

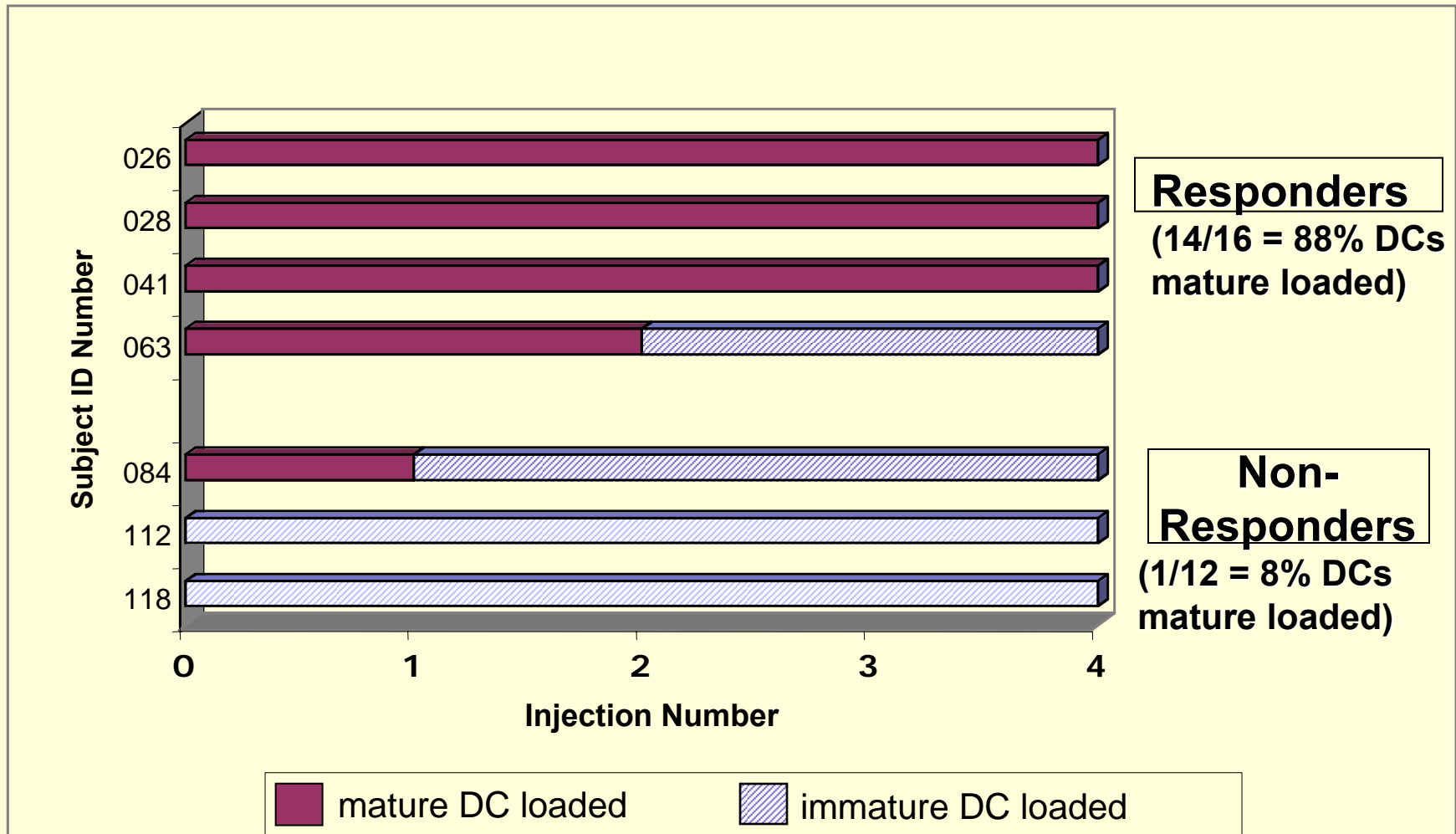


# Pattern noted to cellular responses

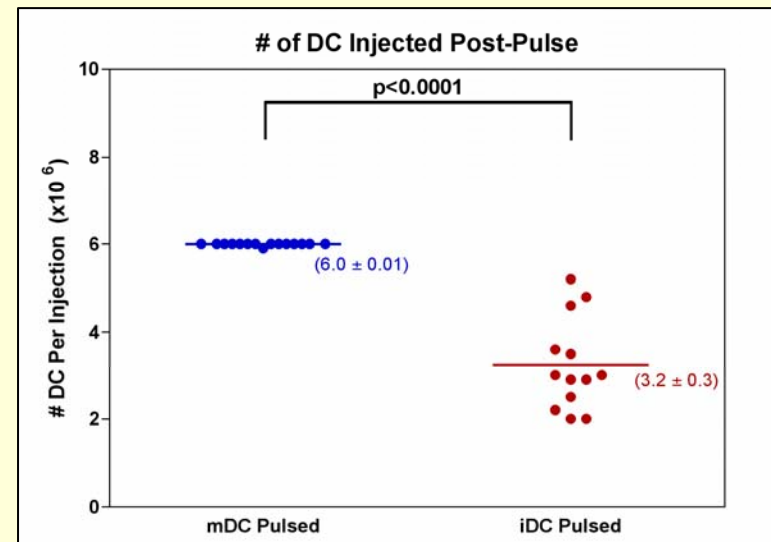
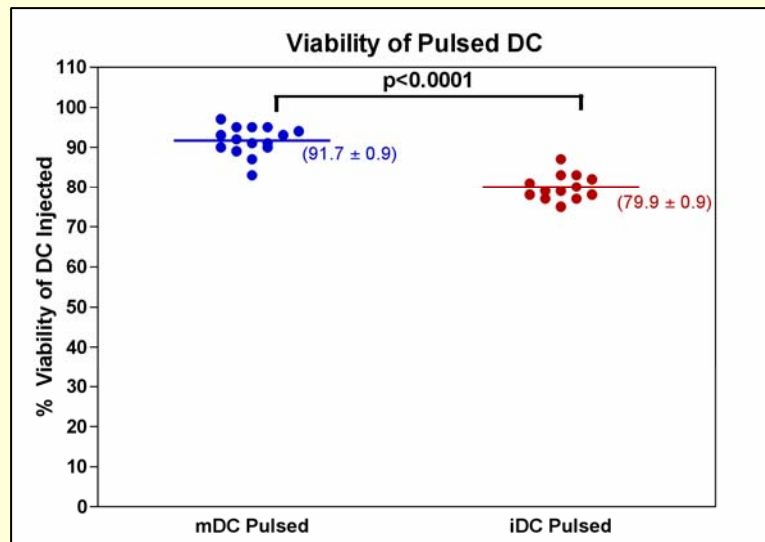
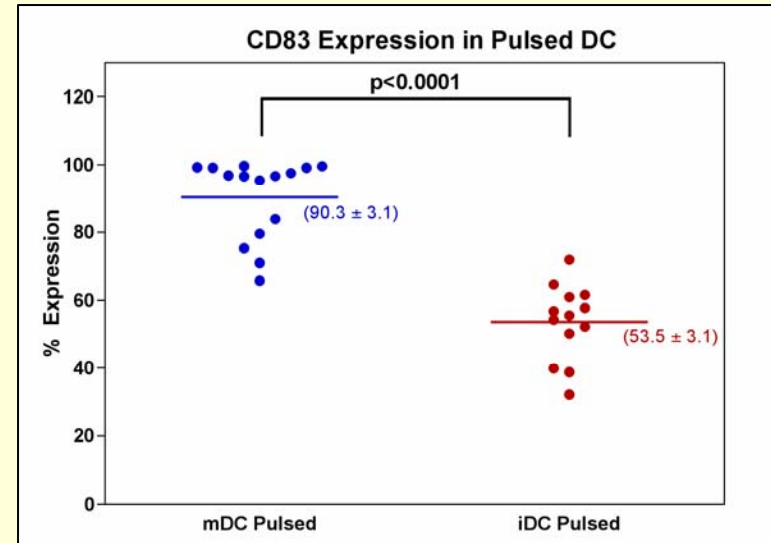
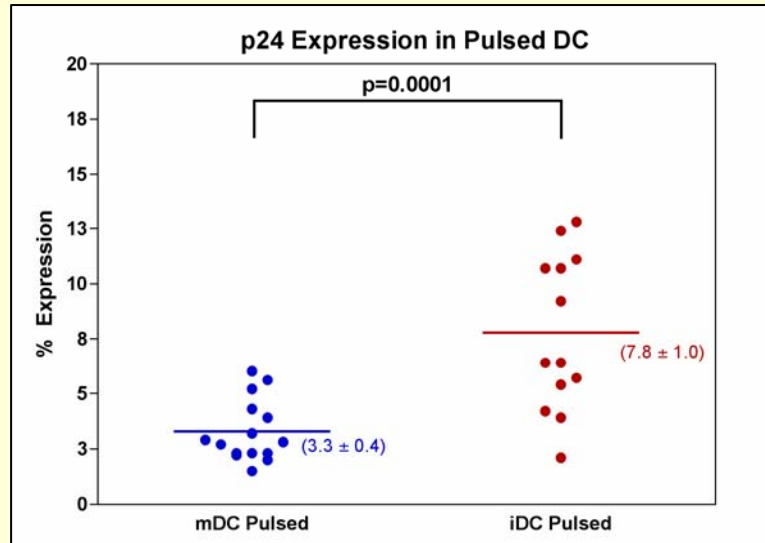
## What changed?

- Pre-study release criteria specified gag expression be  $\geq 5\%$
- After 14 vaccinations (4 vols) with mean gag expression = 4%
- The loading sequence was changed
- Loaded immature DC with vaccine, then matured overnight (same maturation signal)
- This change doubled gag expression (8%) but presented technical challenges that impacted yield, maturity and possibly immune response

# DC loading sequence changed for better transgene expression



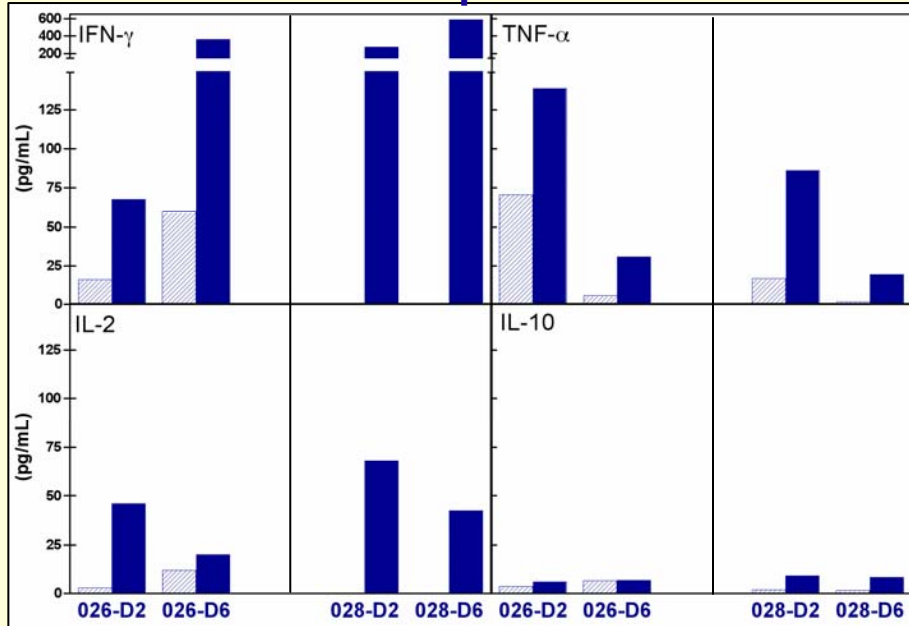
# Loading sequence effects on DC vaccines: immature versus mature loaded



# TNF- $\alpha$ , IL-2 and IFN- $\gamma$ detected after AT-2 stimulation

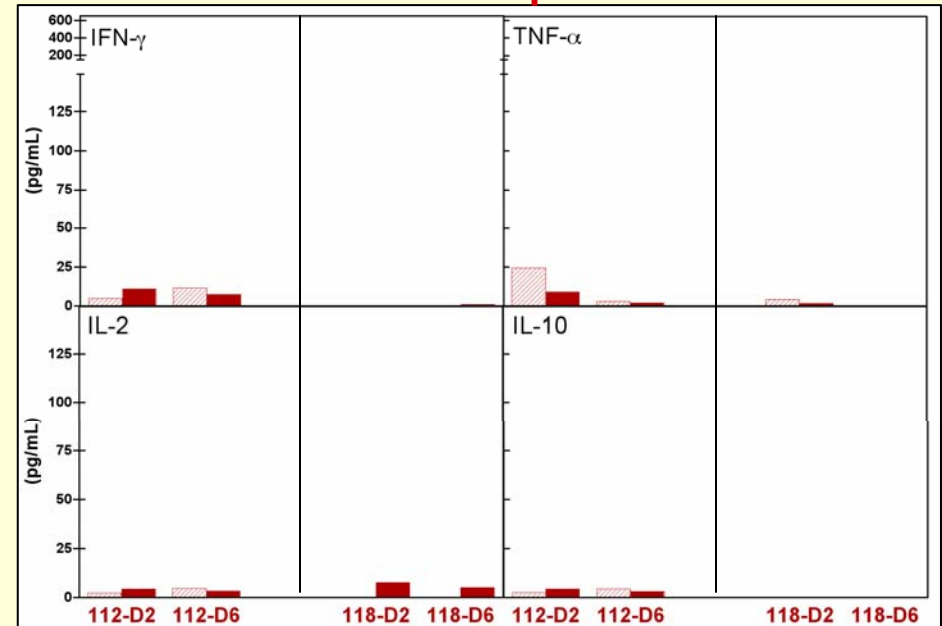
LPA supernatants from 4 donors; samples harvested 2, 6 days after setup

## Responders



Pre-Vaccination  
Post-Vaccination

## Non-Responders

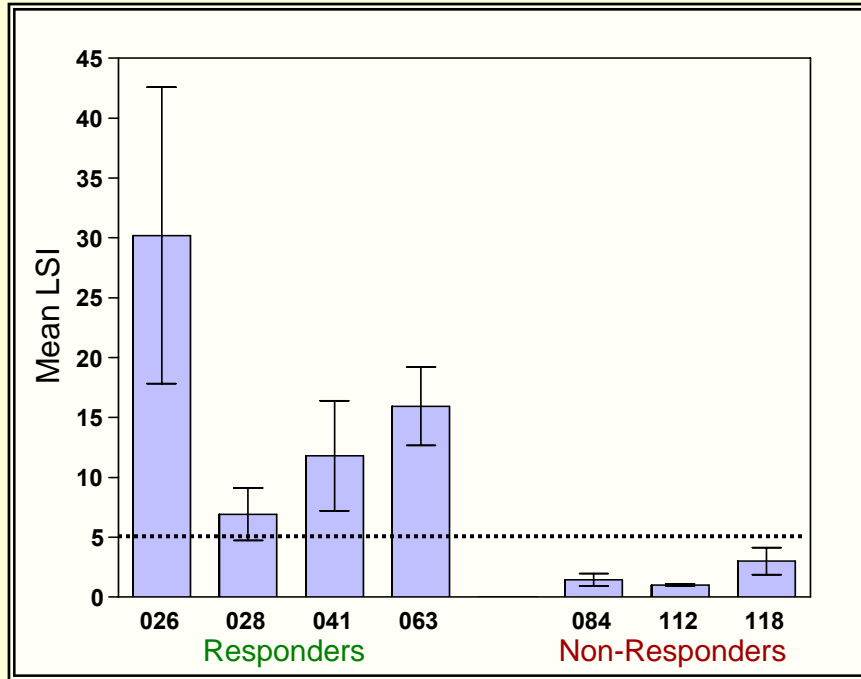


Pre-Vaccination  
Post-Vaccination

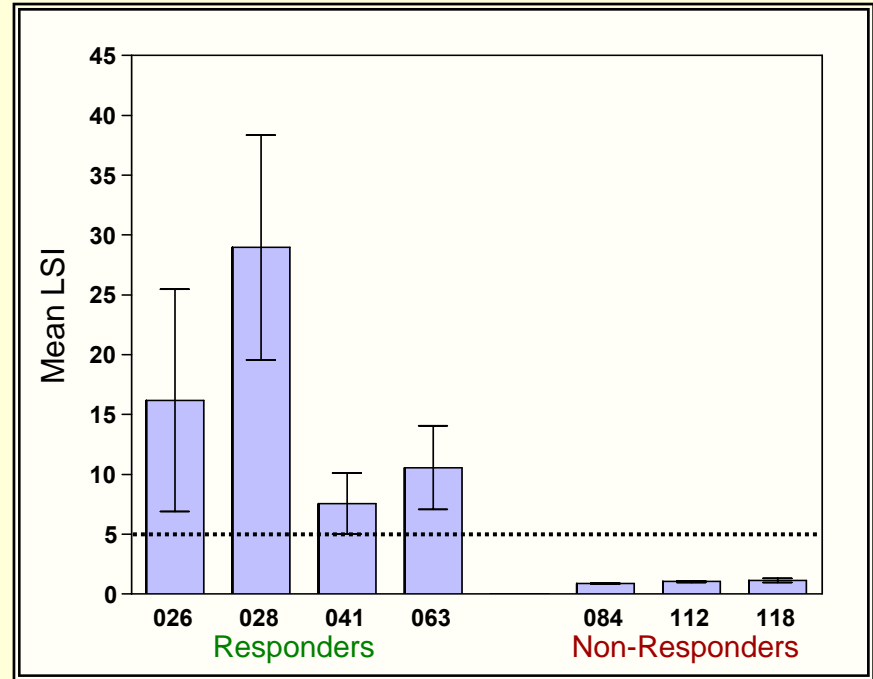
**Cytokines only detected in responders**

# KLH response associated with HIV Immune response

Mean KLH responses (n ≥ 9 visits)



Mean AT-2 responses (n ≥ 7 visits)



**All DC responders respond to KLH-  
no KLH response in DC non-responders  
⇒ suggests DC integrity**

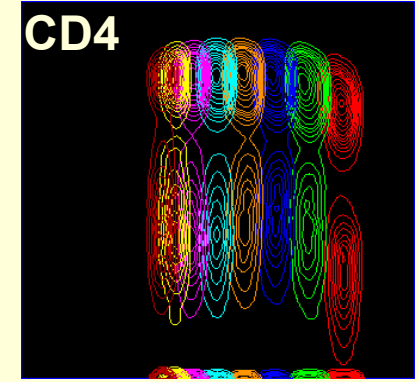
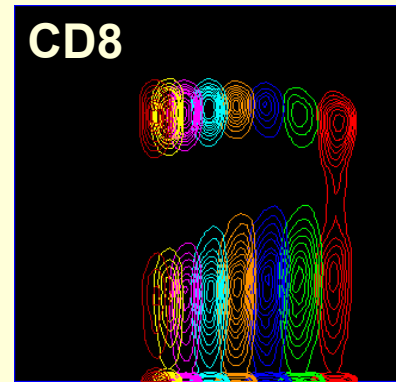
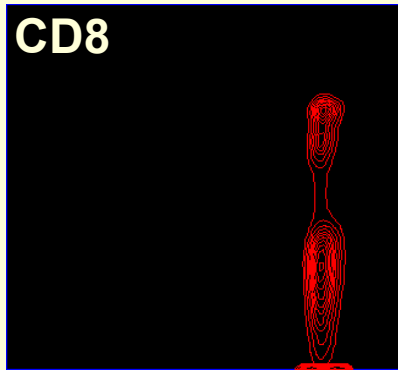


# CFSE shows AT-2 induced CD4 and CD8 Lymphocyte Proliferation

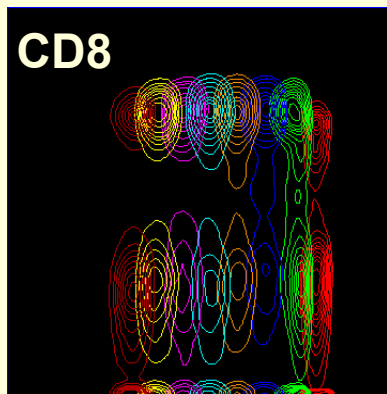
## Pre-Vaccination

### AT-2

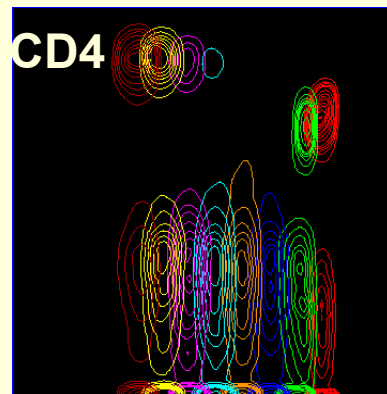
### PHA



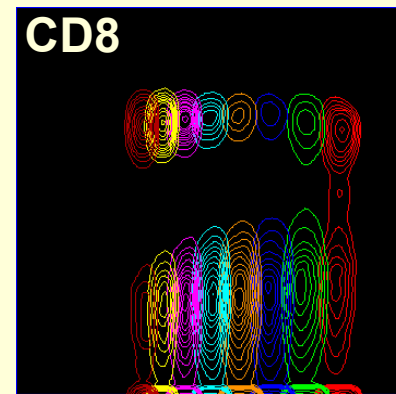
## Post-Vaccination



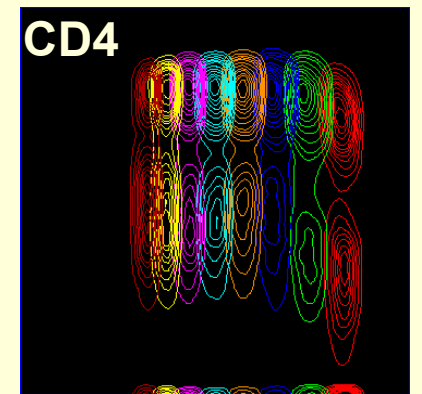
CFSE



CFSE



CFSE



CFSE

# Microarray Analysis

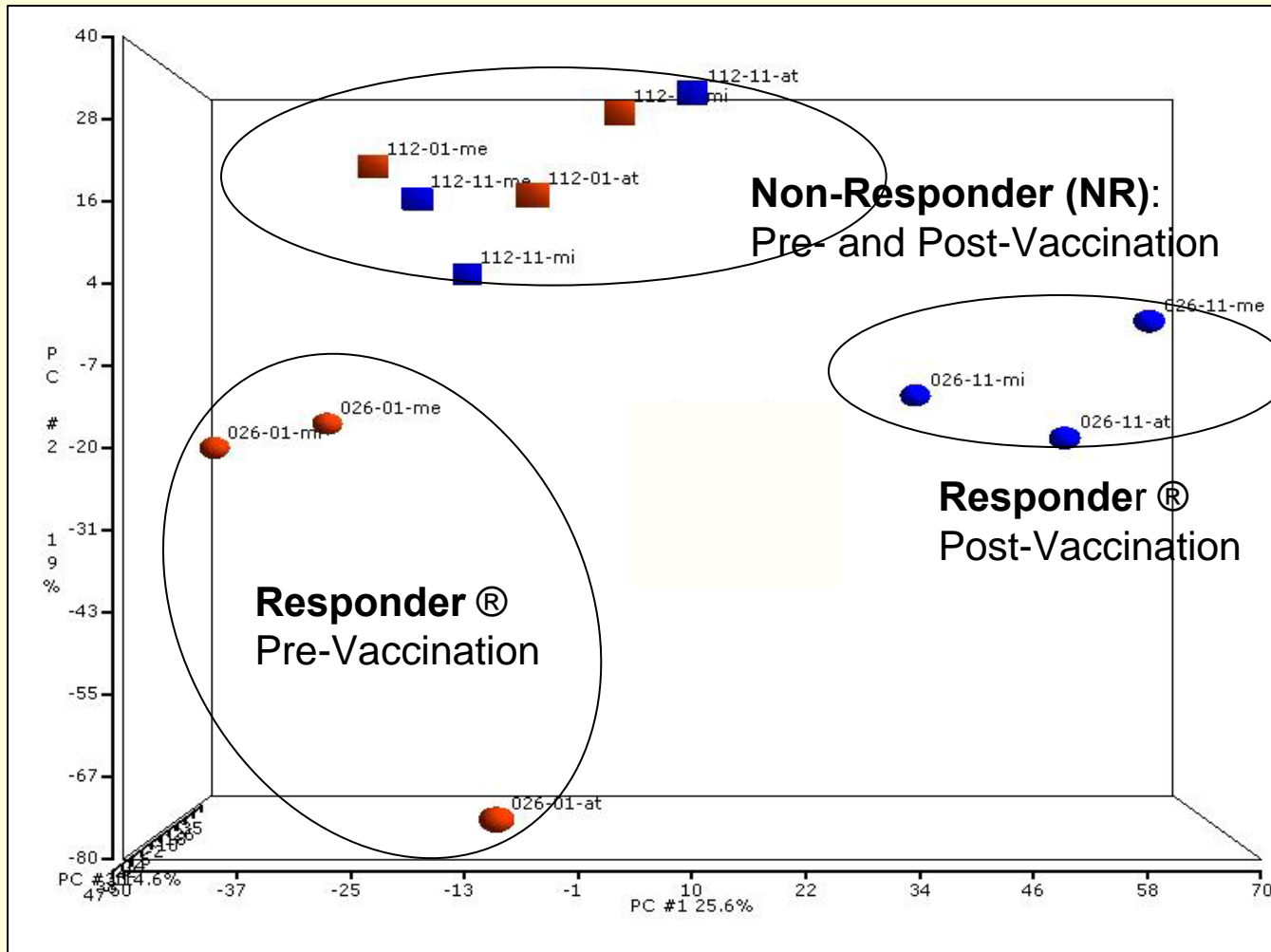
## Experimental Design:

- $10^6$  PBMC from pre- and post-vaccination timepoints from DC-arm vaccine Responders and Non-Responders
- Stimulated 20hrs in 100ng/mL AT2-HIV, equivalent microvesicle control, or media alone
- Cells were harvested, pelleted and flash-frozen
- RNA was extracted, reverse transcribed, labeled with biotin and hybridized to a Affymetrix Human Focus chip
- Data analyzed with bio-informatics tools and algorithms



# Principal Components Analysis - Pilot Experiment

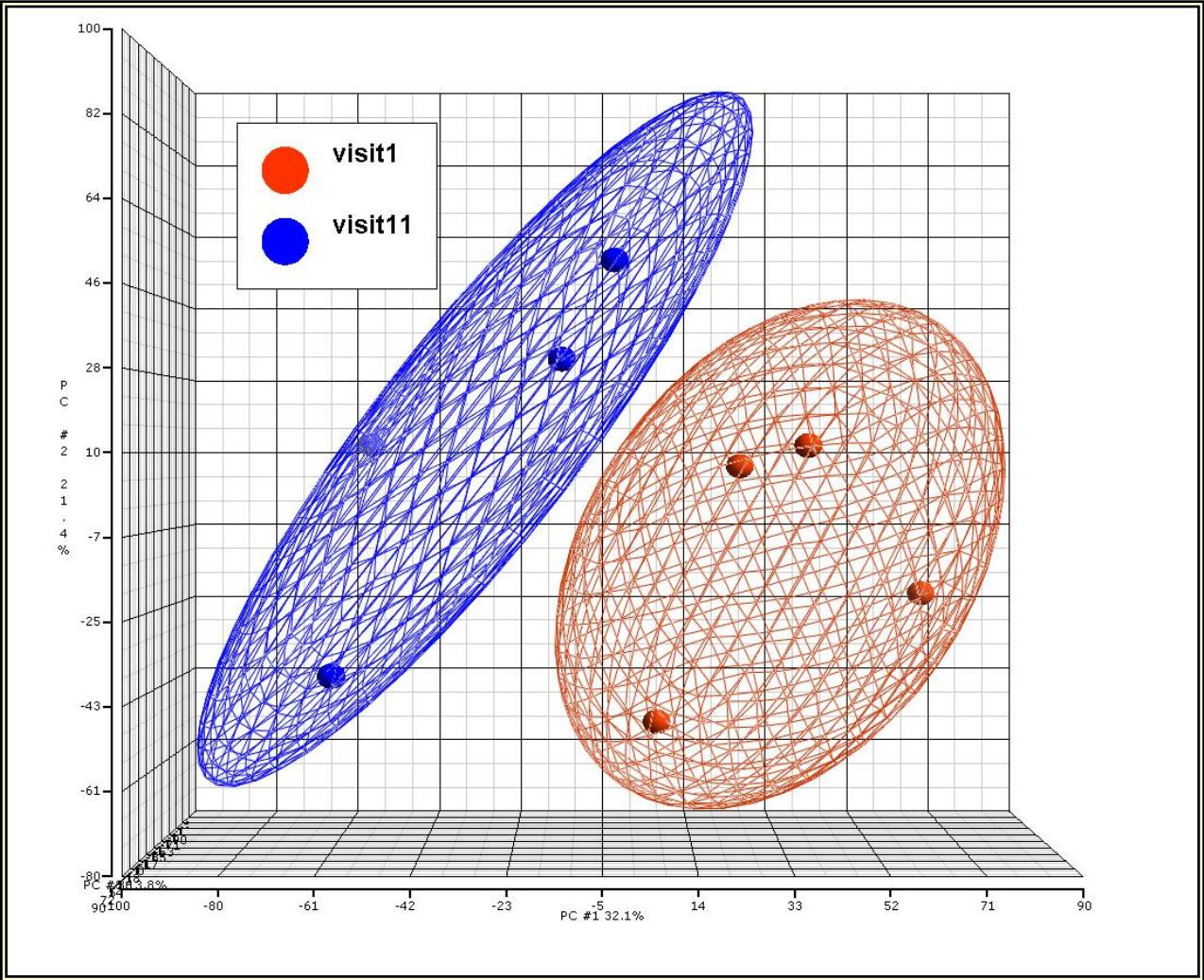
## Data from one representative Responder vs. one Non-Responder



There are significant differences between **NR** and **R**  
And importantly, differences between pre-and post vaccination in the responder samples.



# Principal Components Analysis of AT2 Responders shows complete separation



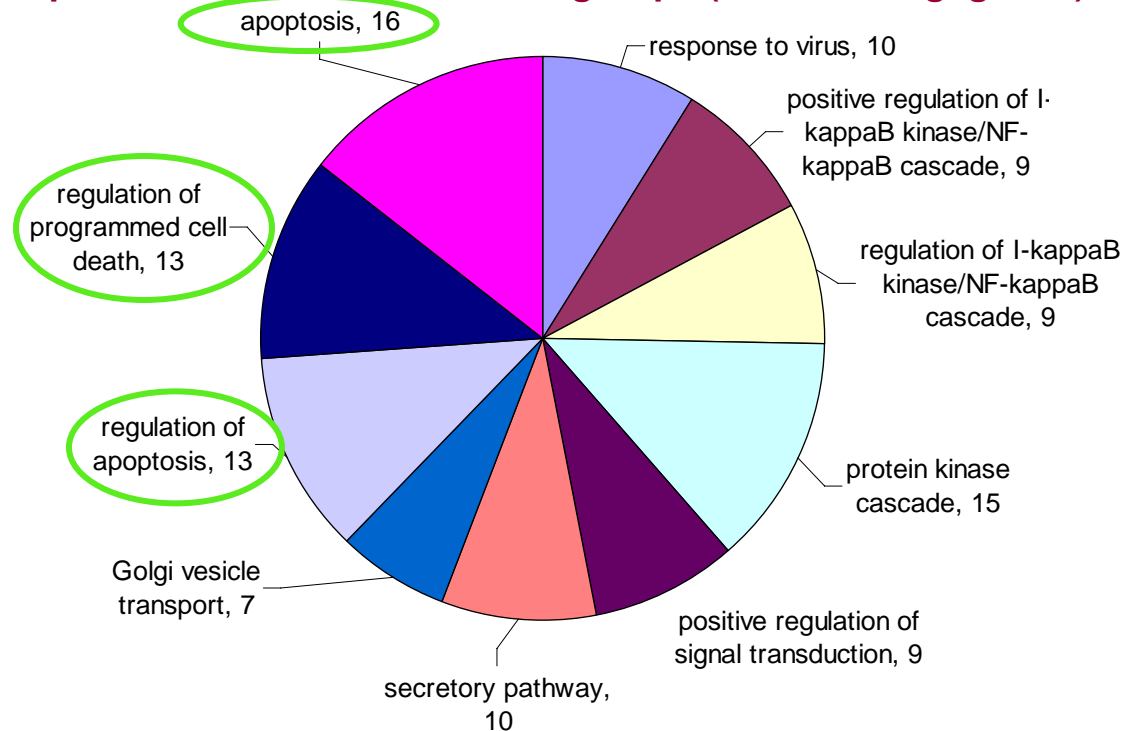
# 205 DEGs identified using highly stringent analysis

- Significance analysis of microarrays (SAM)
- Using the stringent False Discovery Rate <1% (FDR)
- Results comparing Pre- vs. Post-Vaccination timepoints within each donor after stimulation with whole inactivated HIV

	# Genes Significantly Differentially Expressed Pre- vs. Post-Vaccination	
	Responders	Non-Responders
AT2-HIV	205 (2 up* / 203 down)	0
MV ctrl	0	0
Media	0	0

\*Up-regulated genes:  
 oligophrenin-1  
 neuropeptide ff receptor-1

**Top 10 Most affected functional groups (203 downreg. genes)**



# Closing comments

DC-targeted vaccine responses associated with:

- Mature DC loading sequence
  - Loading mature DC may be optimal
  - Loading immature DC may 'impair' the DC itself (load dependent?)
- KLH response
- Increased breadth, reproducible and durable (fresh and frozen)
- Interferon- $\gamma$ , TNF- $\alpha$ , and IL-2 cytokine secretion
- CD4 and CD8 proliferation
- Minimal Ab responses against HIV inserts in DC arm

Program committed to use expression analysis to derive novel correlates of functional immune responses to vaccinations



# Acknowledgements

## WRAIR DC Lab Team

M. Eller, B. Slike  
D. Thelian, J. Anderson  
B. Tassaneetrithep

## Clinical Team

MJ Humphries, K. Duffy  
Y. Lewis, M. Robb, S. Lecher

## Immunology

J. Cox, T Van Cott  
V. Polonis

## Genomics

M. Vahey, E. Lesho

## WRAIR-Retrovirology

N. Michael, D. Birx

## Aventis Pasteur

R. El-Habib  
S. Gurunathan  
C. Meric

## SAIC-Frederick

L. Arthur, J. Lifson

## Rockefeller University

M. Dhodapkar, N. Bhardwaj  
S. Schlesinger, R. Steinman,

