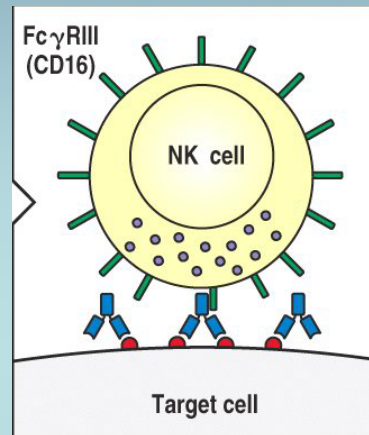


# NK mediated Antibody Dependent Cellular Cytotoxicity in HIV infections



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MELBOURNE

# ADCC process consists of

QuickTime™ and a  
TIFF (Uncompressed) decompressor  
are needed to see this picture.

1. Target cells expressing pathogen's antigen on the surface

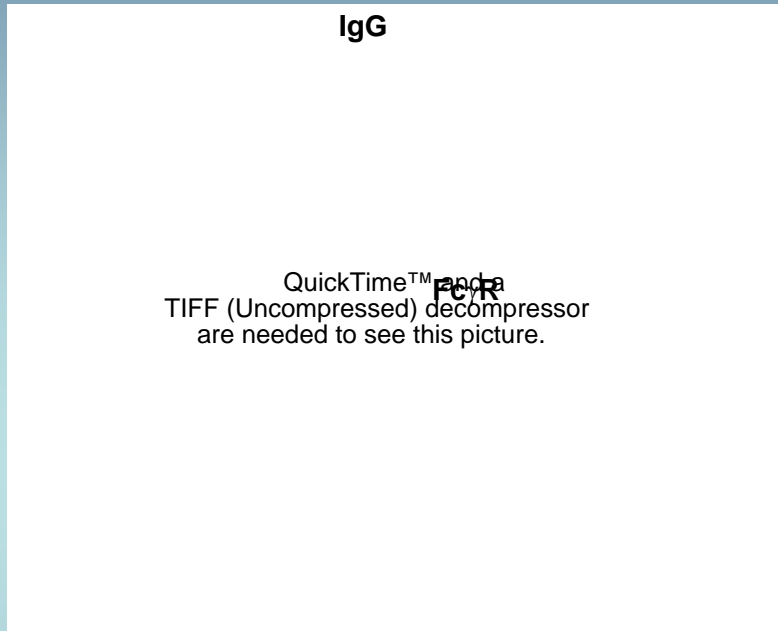
# ADCC process consists of

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**IgG Ab**

1. target cells expressing pathogen's antigen on the surface
2. Antibodies of IgG isotype to target antigen

# ADCC process consists of



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2. Antibodies of IgG isotype to target antigen
3. Effector cells (NK cells) with Fc $\gamma$ R (gamma receptor)

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Ag

1. target cells expressing pathogen's antigen on the surface
2. Antibodies of IgG isotype to target antigen
3. Effector cells (NK cells) with Fc $\gamma$ R (gamma receptor)

- **Collaboration between innate (NK cells) and adaptive (Ab) immune response**

- **NK cells kill cells in the absence of MHC while having high specificity mediated by Antibodies**

# Importance of ADCC against HIV

- HIV Human Cohort studies:
  - Low ADCC titres correlate with progression and CD4+ cell decline *Baum 1996, Forthal 2001,*
- Macaque Cohort Studies:
  - Animals with higher ADCC responses progress more slowly *Banks 2002, Ohkawa 1994*
- Macaque Vaccine Studies:
  - Vaccine elicited ADCC activity correlates with reduced viral load *Gomez-Roman 2005, Florese 2006,*
- Monkey Passive Transfer Study:
  - Abrogating Fc binding substantially reduces efficacy of Nab *Hessell 2007*

# Novel ICS Assay to detect ADCC

HIV positive human whole blood +  
gp140 protein or  
overlapping HIV peptide pools  
(15 aa overlapping by 11 aa)

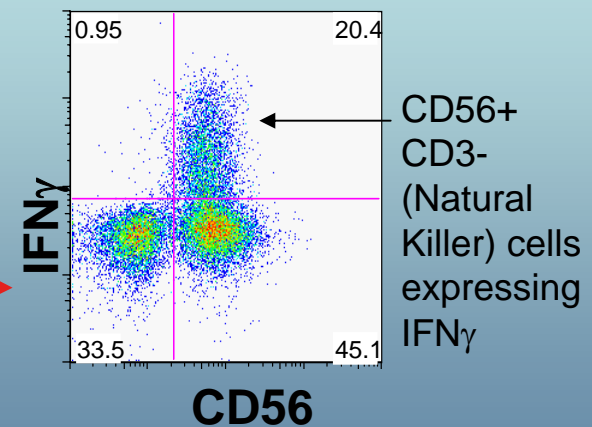
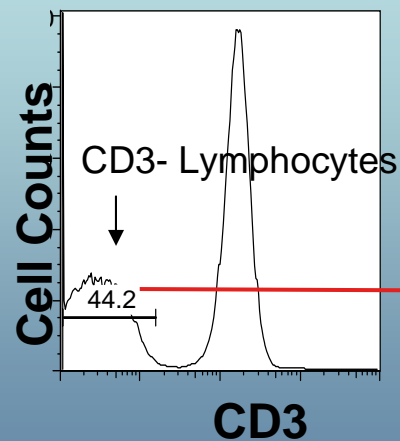
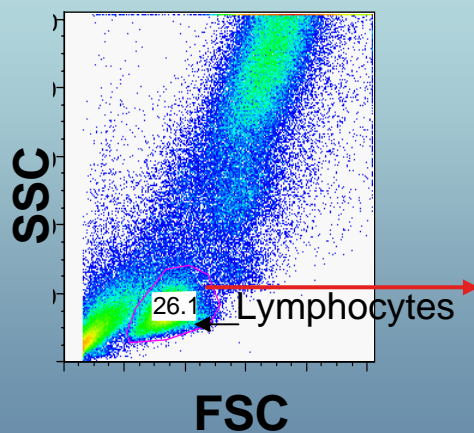
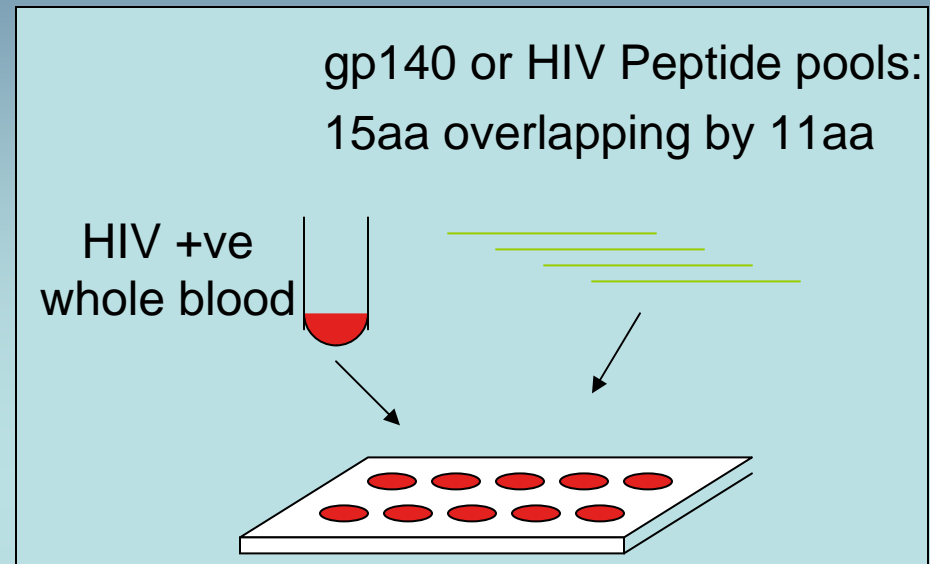
Brefeldin A

Incubate 37° 5% CO<sub>2</sub>

Stain for CD2, CD56, CD3

Stain for intracellular IFN $\gamma$

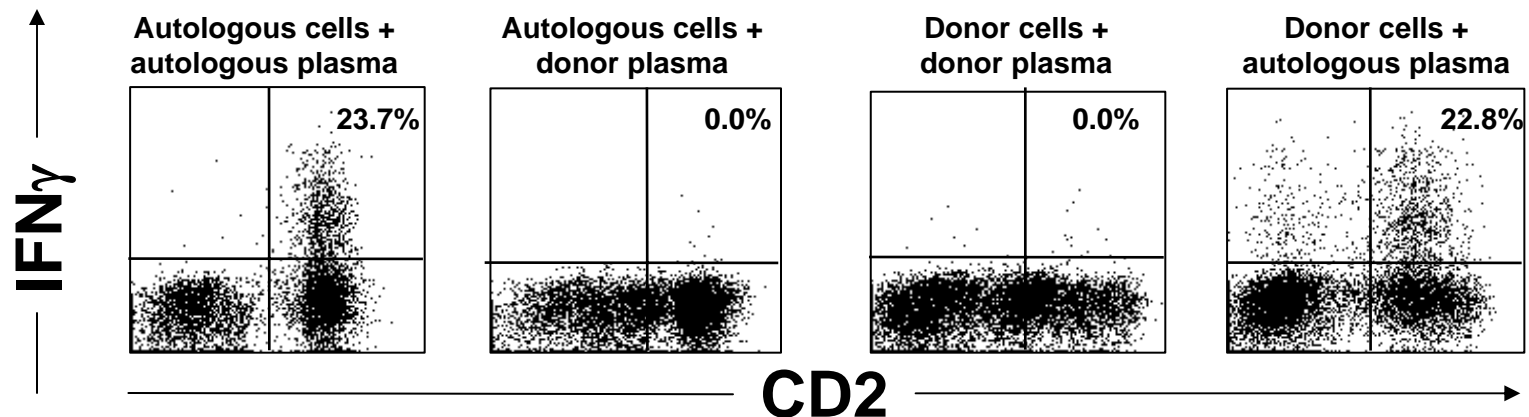
Acquire cells on FACS



# IgG mediates NK cell activation in ICS Assay to detect ADCC

- HIV serum mediated
- Specifically IgG mediated
- Not complement mediated
- Does not require co-stimulation
- Readily maps linear ADCC epitopes
- Can phenotype response
- Measures multiple effector functions

Gated on CD3- lymphocytes





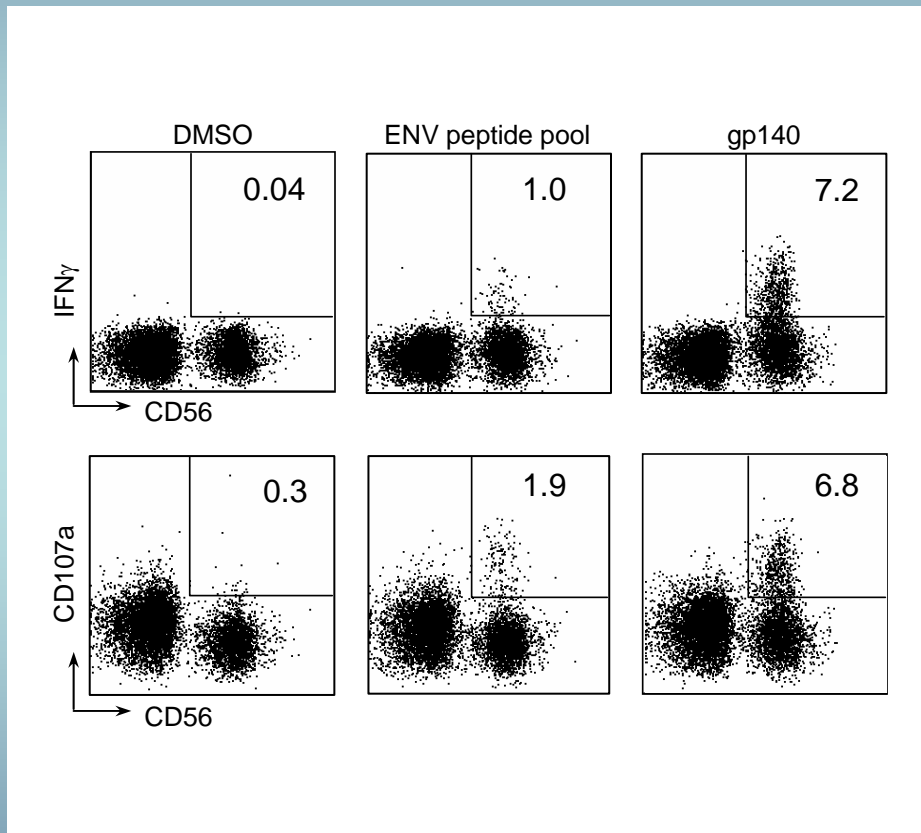
# Phenotype of NK effector cells in ADCC ICS Assay

Cell Surface Marker	Expression
CD3	-
CD4	-
CD8	-
TCR $\alpha\beta$	-
TCR $\gamma\delta$	-
} <b>T cell markers</b>	
CD19	-
CD20	-
} <b>B cell markers</b>	
CD2	+
CD16	+/-
CD56	+/-
CD85j	+/-
CD94 (KLRD1)	+/-
CD158a (KIR2DL2)	+/-
CD161 (KLRB1)	+/-
NKB1 (KIR3DL1)	+/-
} <b>NK cell markers</b>	

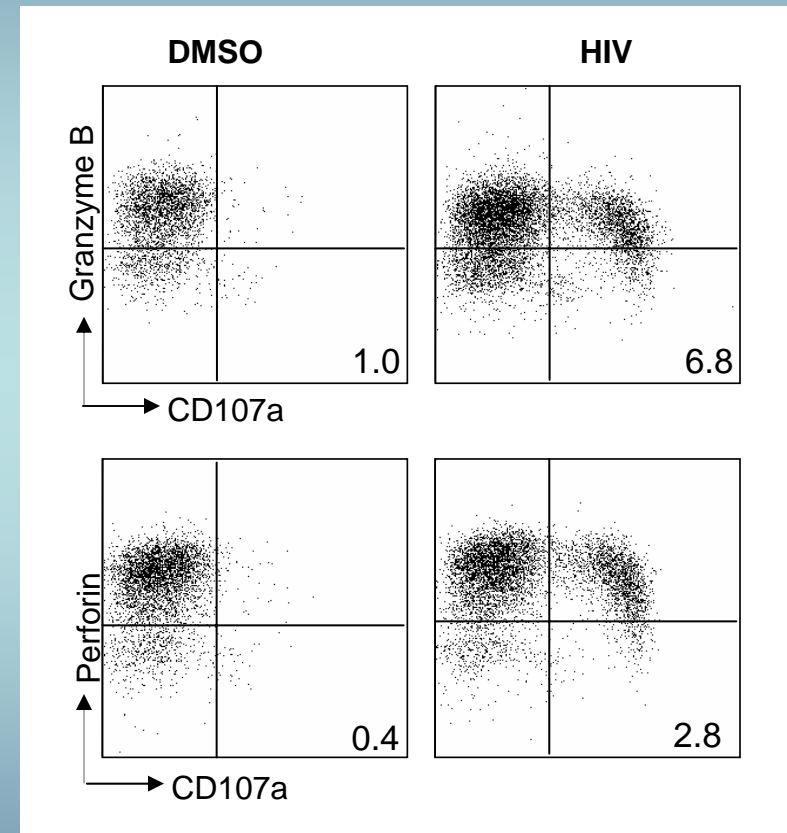
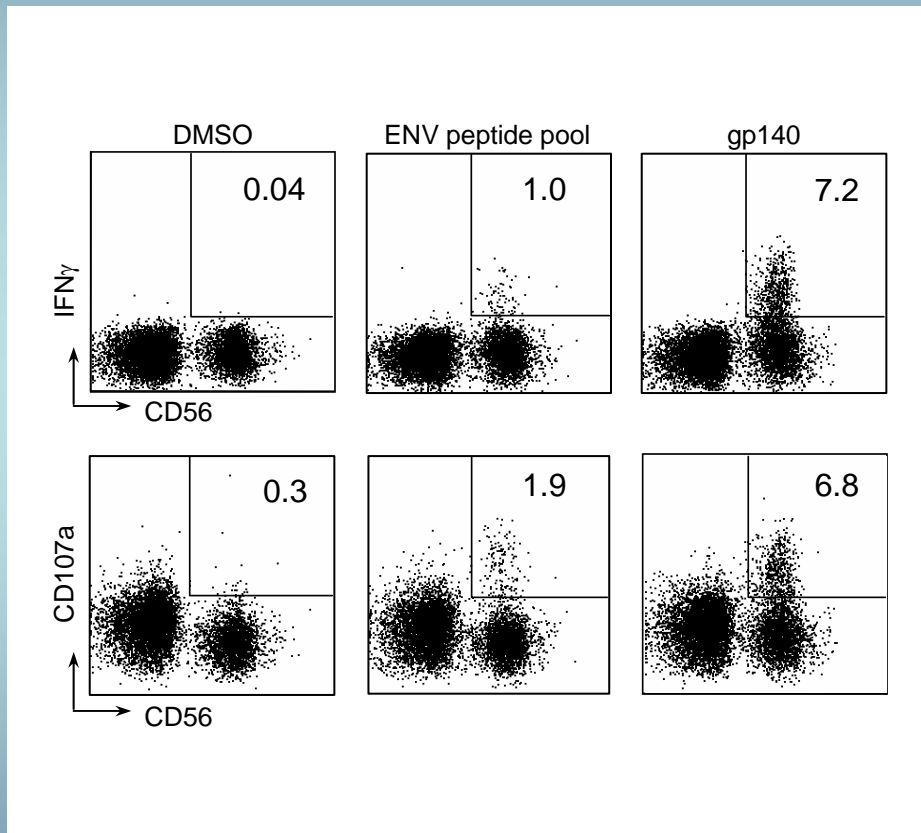
Effector Molecule	Expression
IFN $\gamma$	+
Granzyme	+
Perforin	+
TNF $\alpha$	+
CD107a	+
IL-2	-
IL-4	-

Effector cells express characteristic NK cell phenotype

# Cytolytic functions of NK cells measured by ADCC ICS Assay



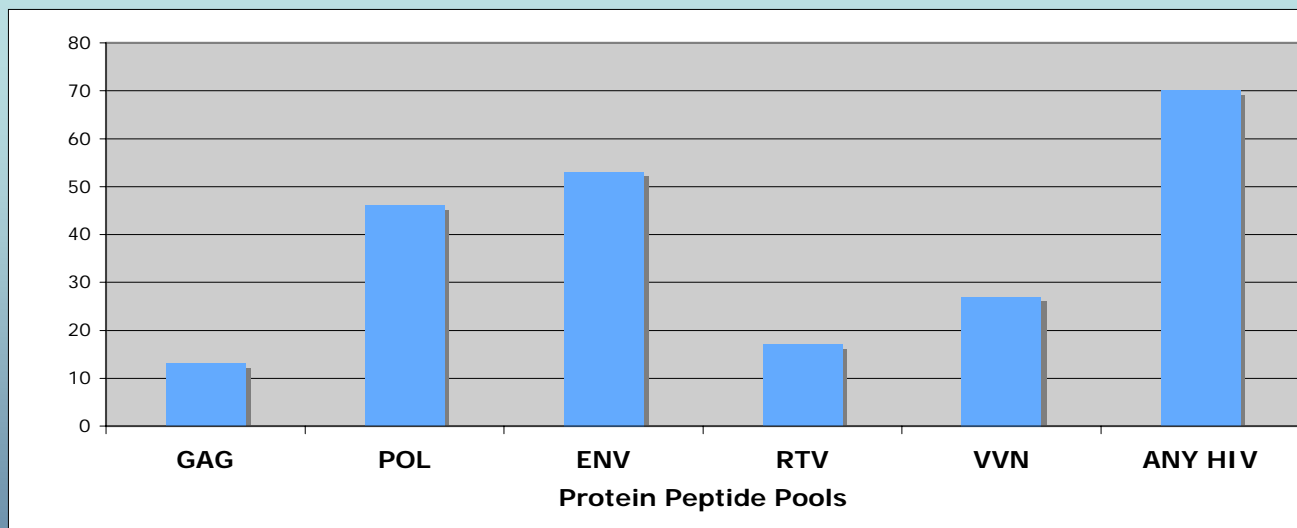
# Cytolytic functions of NK cells measured by ADCC ICS Assay



# Recruit ADCC Cohort

- Currently recruited 80 ART naïve subjects
  - ADCC responses measured by ICS Assay

Protein pool	GAG	POL	ENV	RTV	VVN	ANY HIV
No. of Cohort	13	46	53	17	27	70
% of Cohort	16%	55%	66%	21%	34%	87%

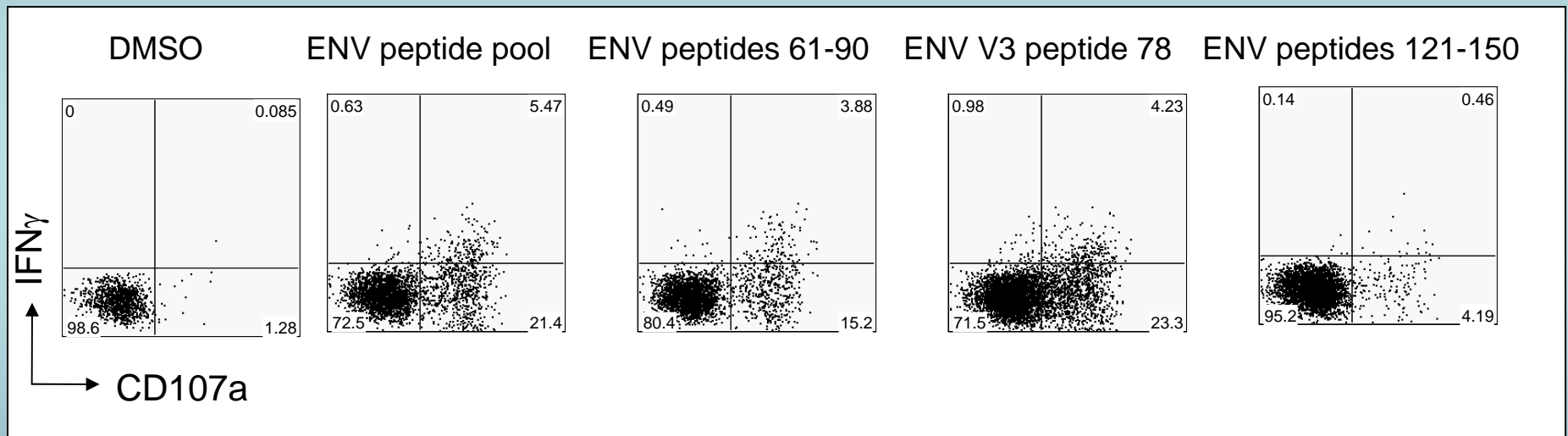


RTV= Rev, Tat, Vpu

VVN= Vif, Vpr, Nef combined peptide pools

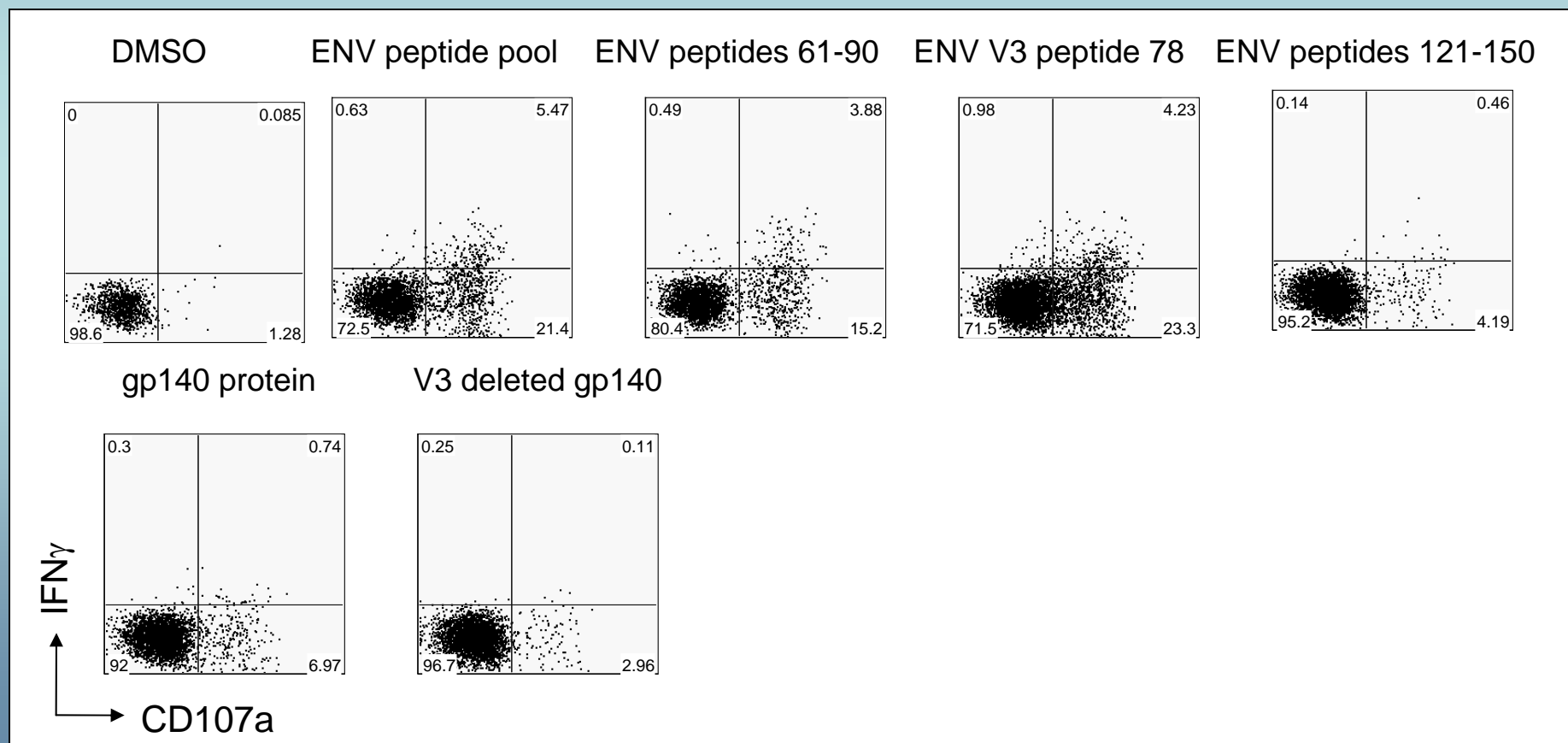
# What epitopes do ADCC target?

- Use of HIV overlapping peptide pools allows us to readily map linear ADCC epitopes



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# Mapping ADCC epitopes on ENV

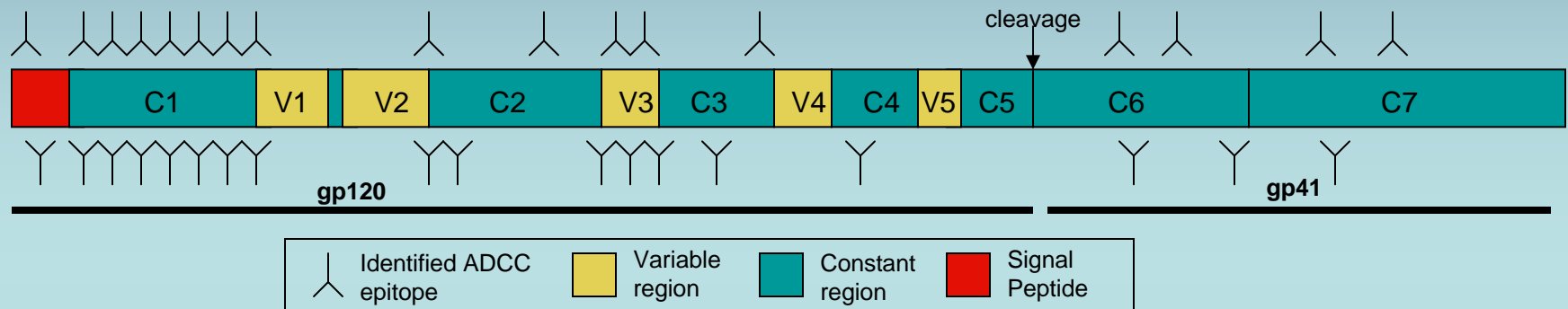


Figure: Map of identified ADCC Epitopes in gp160 Envelope glycoprotein

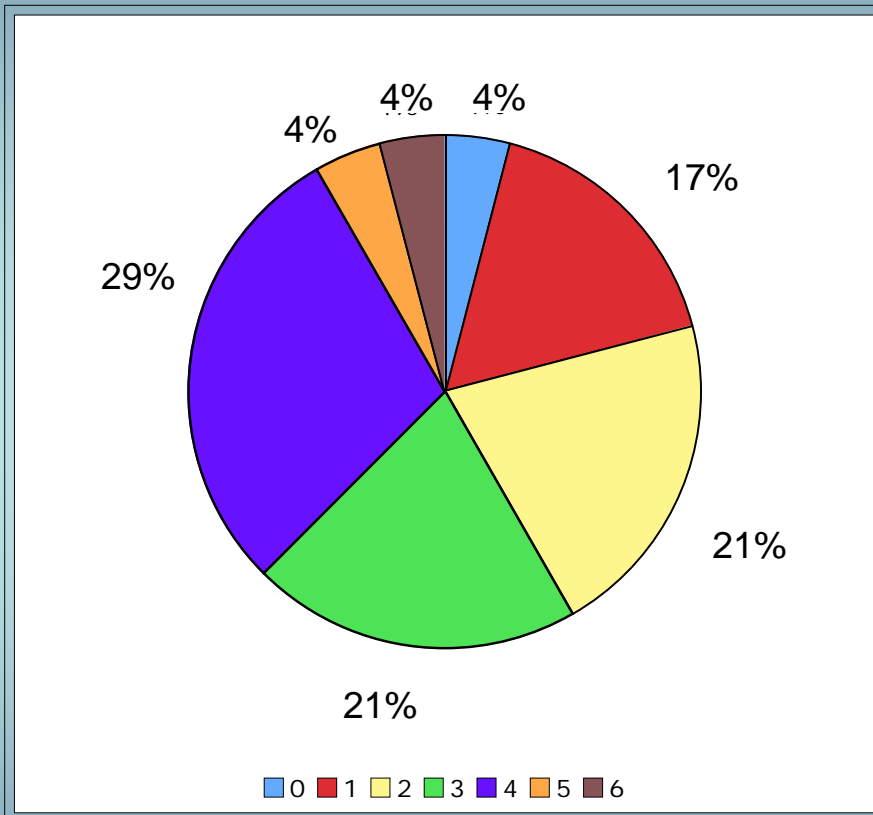
- Identified 37 novel ENV specific linear ADCC epitopes
  - 9 epitopes shared by 2 or more subjects
- Additional epitope in VPU mapped
- Currently mapping additional Pol epitopes

# Do these ADCC responses force immune escape?

- Investigate the possibility of viral mutational escape from ADCC activity
  - See if ADCC exerts significant immune pressure upon the virus



# Viral sequence variation at ADCC epitopes



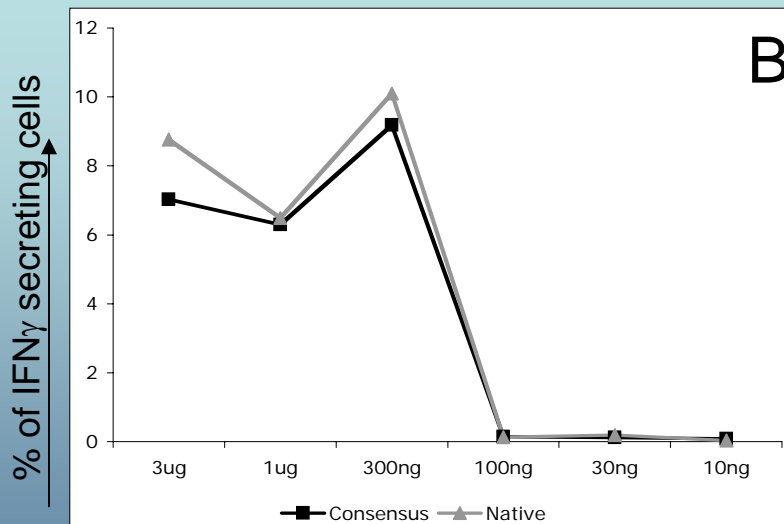
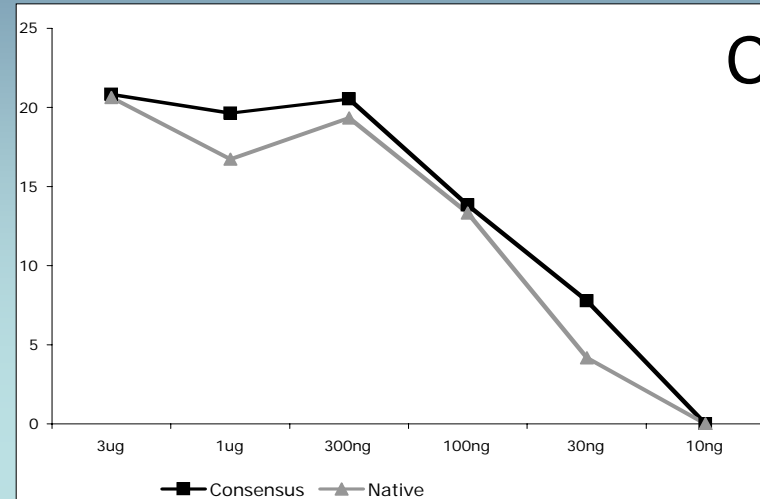
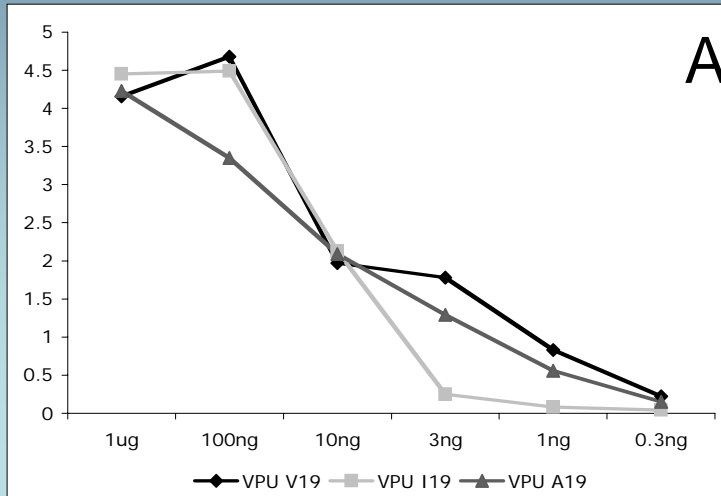
Number of amino acid changes within epitope sequence in autologous native virus compared to consensus B HIV

## Investigated biological relevance of mapped ADCC epitopes

Cloned and sequenced several subjects' concurrent autologous plasma virus samples across the mapped ADCC epitopes and identified variations from HIV-1 consensus sequence

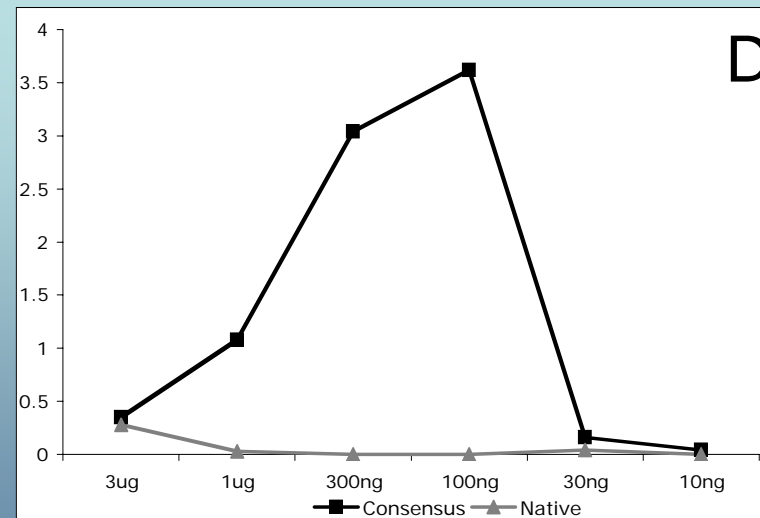
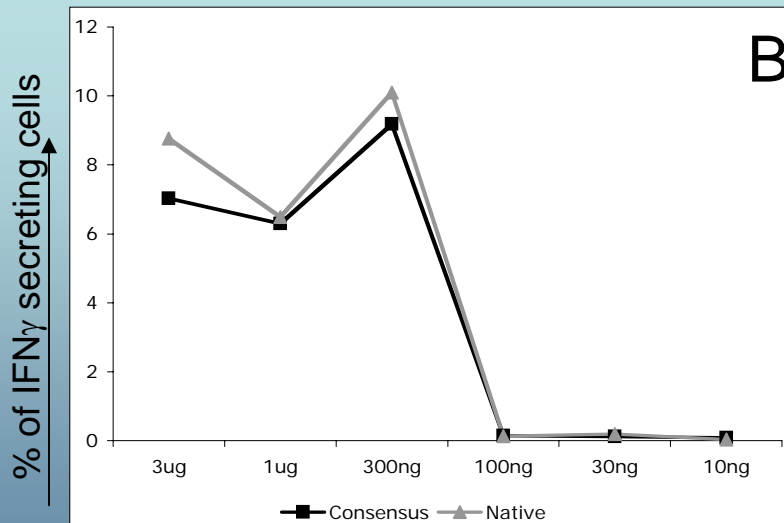
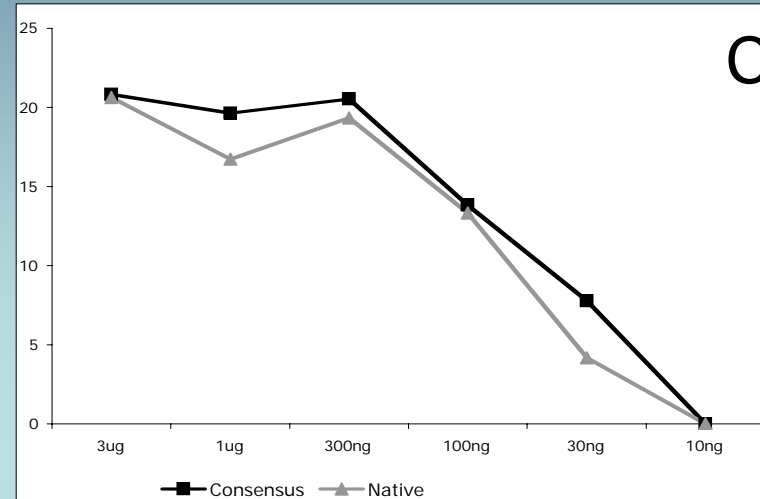
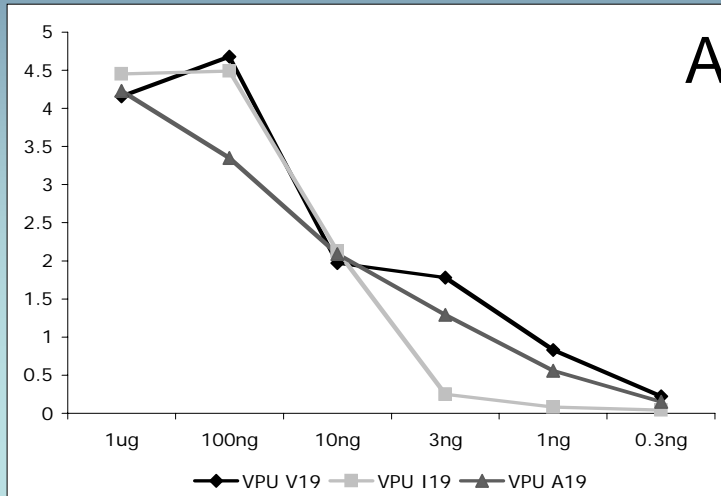
## Identified sequence variation in 96% of ADCC epitopes

# ADCC escape



Concentration of peptide per 200ul well

# ADCC escape



Concentration of peptide per 200ul well

# Future Aims

- Functionally compare more consensus and autologous ADCC epitopes
- Conduct a prospective and longitudinal study of ADCC from HIV cohort
  - Identify ADCC epitopes associated with slow progression of disease
- Reach a clearer understanding of significance of ADCC responses in HIV infection

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Kent Lab



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