

# Reducing complexity of multidimensional data

## Applications on polyfunctional T cell immunology

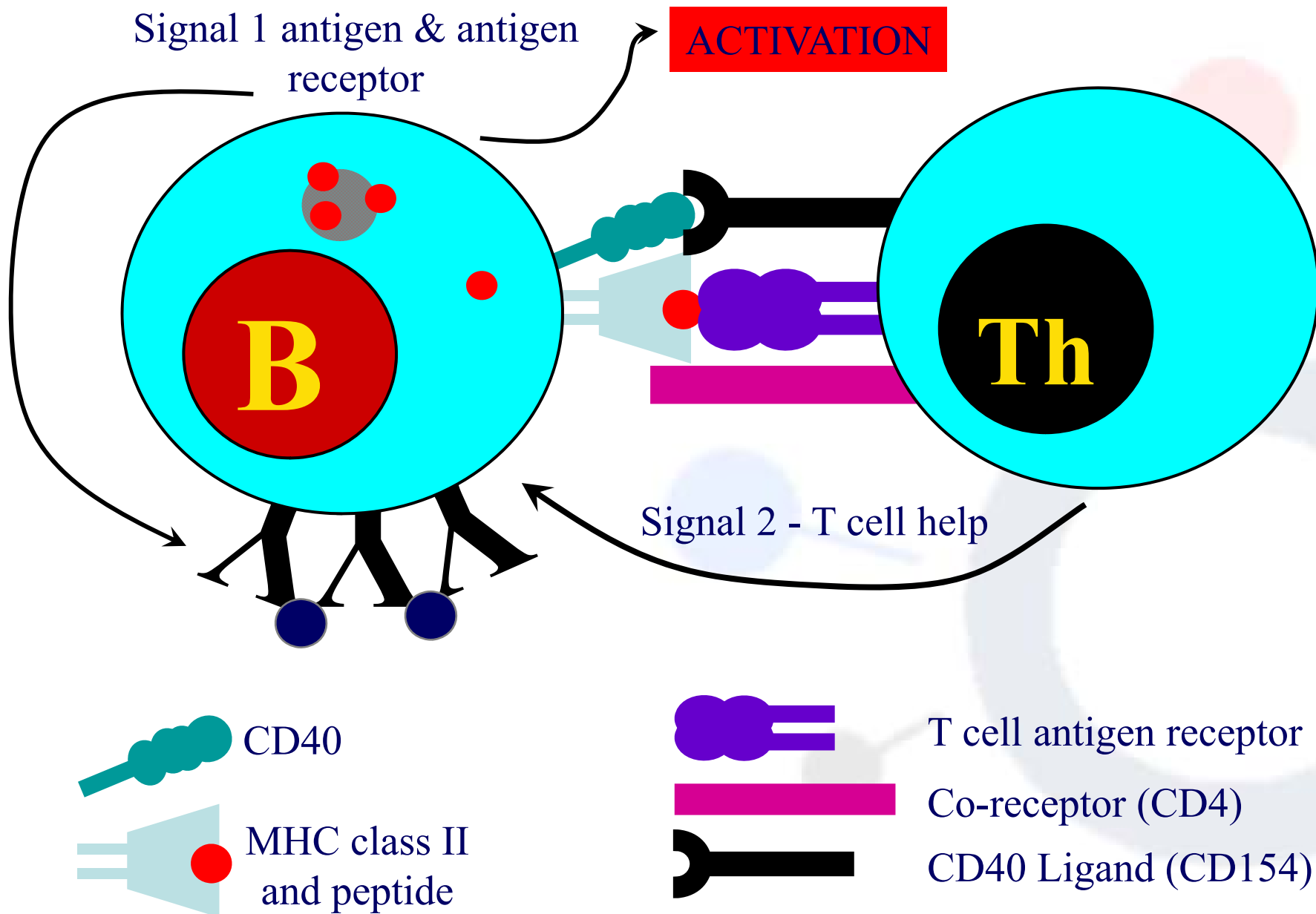
**Martin LARSEN**

[www.Immulab.fr](http://www.Immulab.fr)

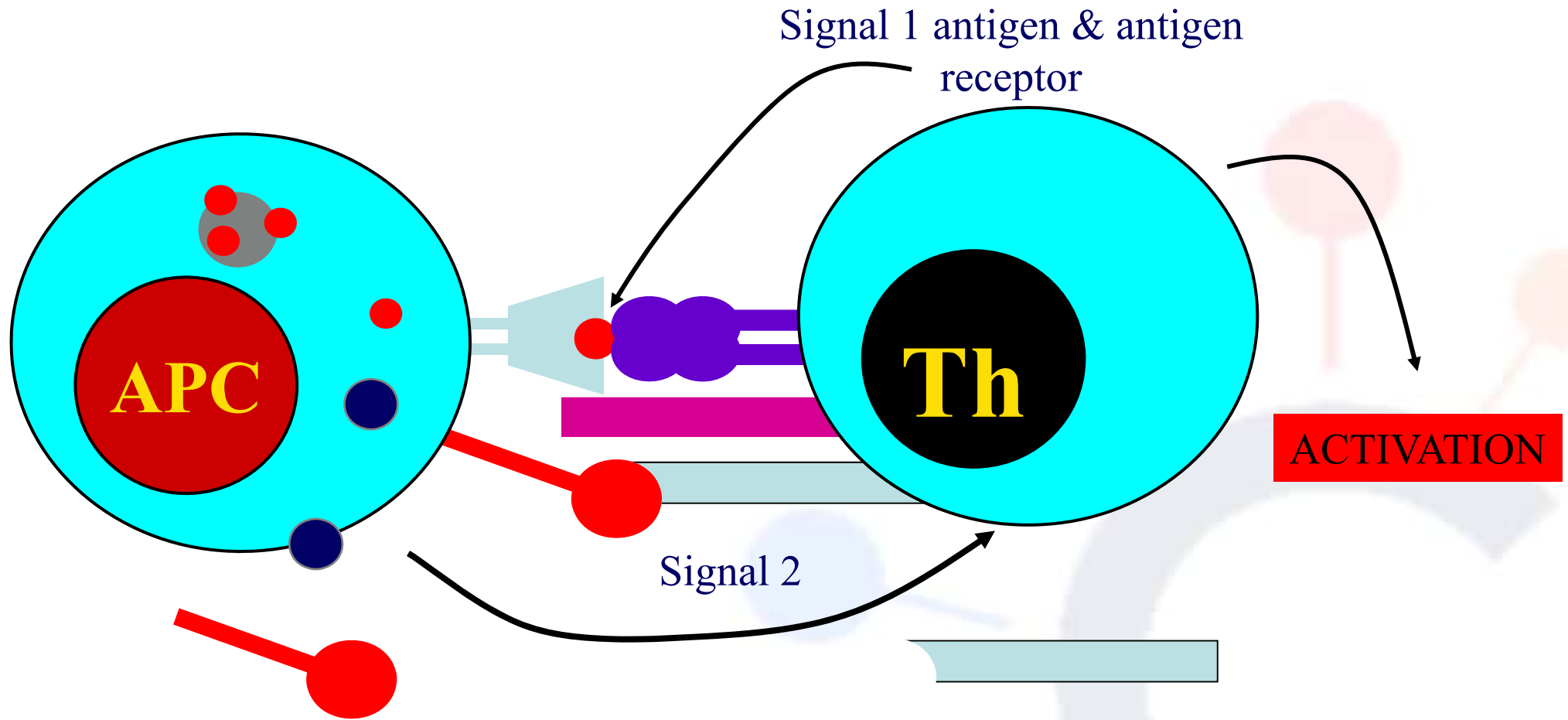
INSERM U1135, CHU Pitié-Salpêtrière, Paris, France

# T helper cells costimulate B cells

## Two - signal models of activation



# Antigen presentation - T cells are co-stimulated

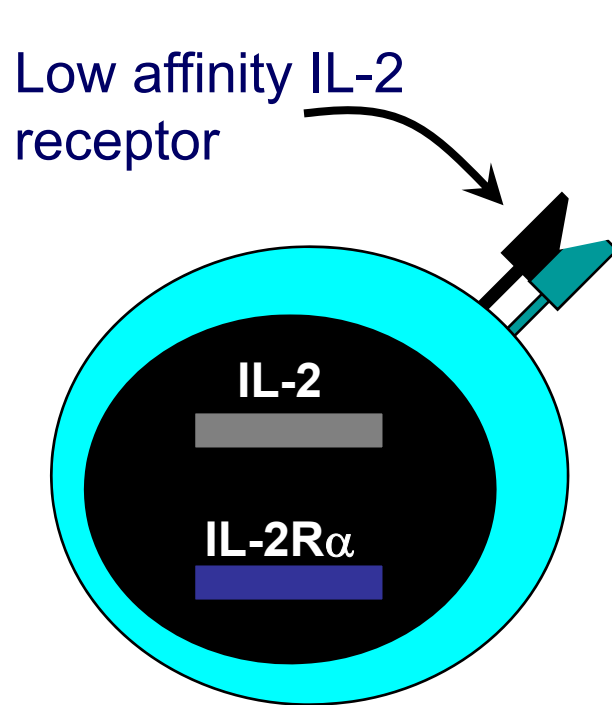


B7 family members (CD80 & CD86)

CD28

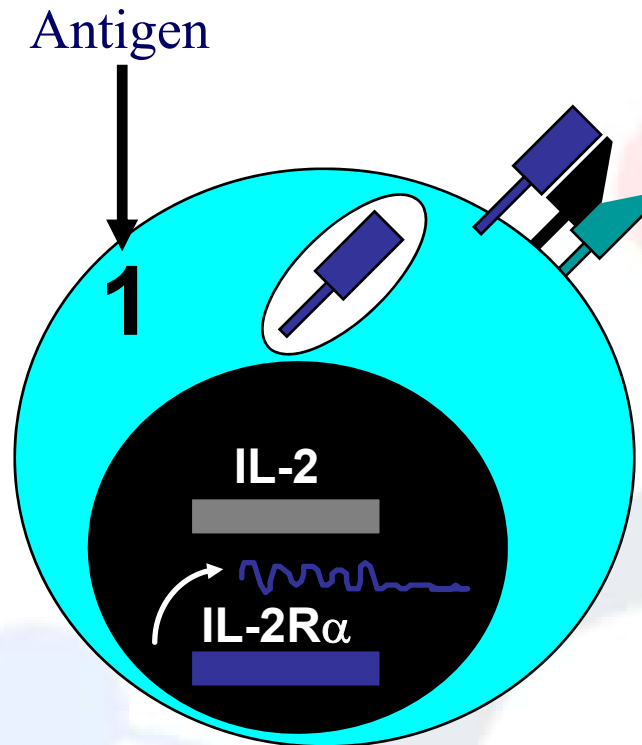
Costimulatory molecules are expressed by most APC including dendritic cells, monocytes, macrophages, B cells etc., but not by cells that have no immunoregulatory functions such as muscle, nerves, hepatocytes, epithelial cells etc.

# Mechanism of co-stimulation in T cells



Resting T cells

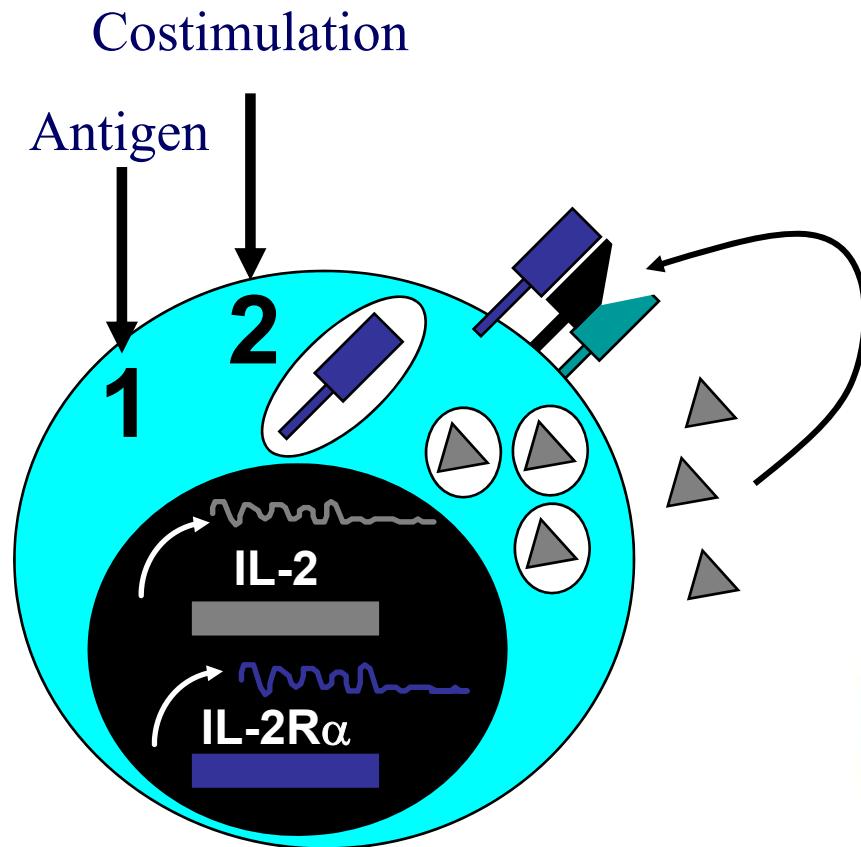
Express IL-2 receptor- $\beta$  and  $\gamma$  chains but no  $\alpha$  chain or IL-2



Signal 1

NFAT binds to the promoter of the  $\alpha$  chain gene of the IL-2 receptor. The  $\alpha$  chain converts the IL-2R to a high affinity form

# Mechanism of co-stimulation in T cells



## Signal 2

Activates AP-1 and NF $\kappa$ -B to increase IL-2 gene transcription by 3 fold

Stabilises and increases the half-life of IL-2 mRNA by 20-30 fold

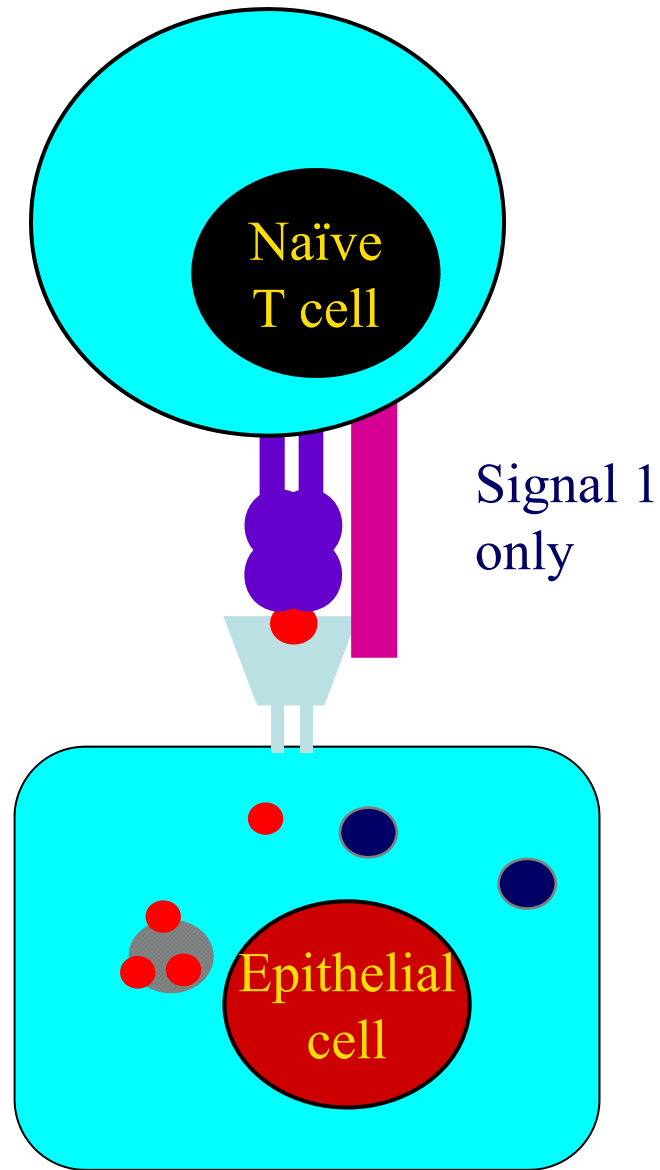
IL-2 production increased by 100 fold overall

Immunosuppressive drugs illustrate the importance of IL-2 in immune responses

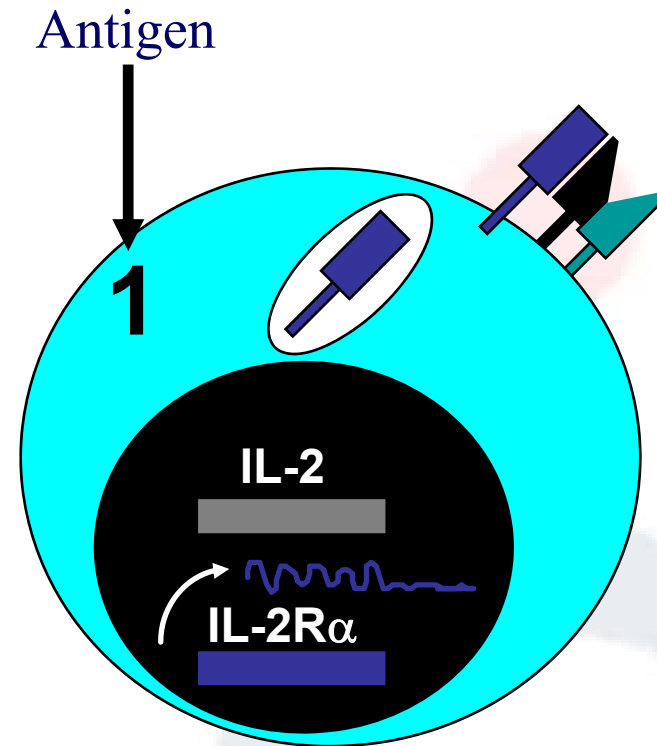
Cyclosporin & FK506 inhibit IL-2 by disrupting TcR signalling

Rapamycin inhibits IL-2R signalling

# Anergy



Self peptide epitopes presented by a non-classical APC e.g. an epithelial cell

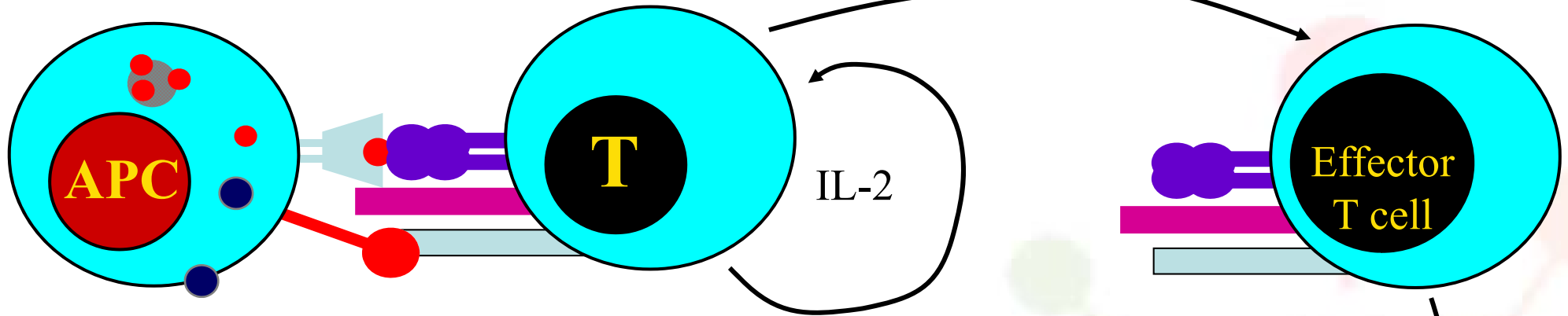


The T cell is unable to produce IL-2 and therefore is unable to proliferate or be clonally selected.

Unlike immunosuppressive drugs that inhibit ALL specificities of T cell, signal 1 in the absence of signal 2 causes antigen specific T cell unresponsiveness.

# Arming of effector T cells

Clonal selection and differentiation



Activation of **NAÏVE** T cells by signal 1 and 2 is not sufficient to trigger **effector** function, but.....

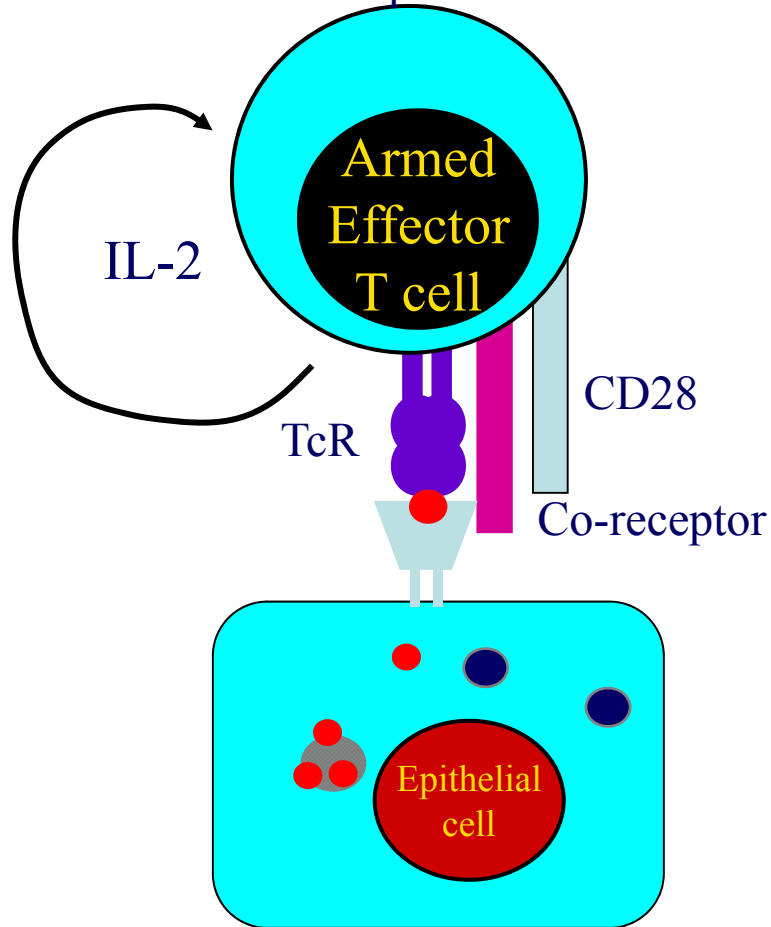
the T cell *will* be activated to proliferate and differentiate under the control of autocrine IL-2 to an effector T cell.

These T cells are **ARMED**

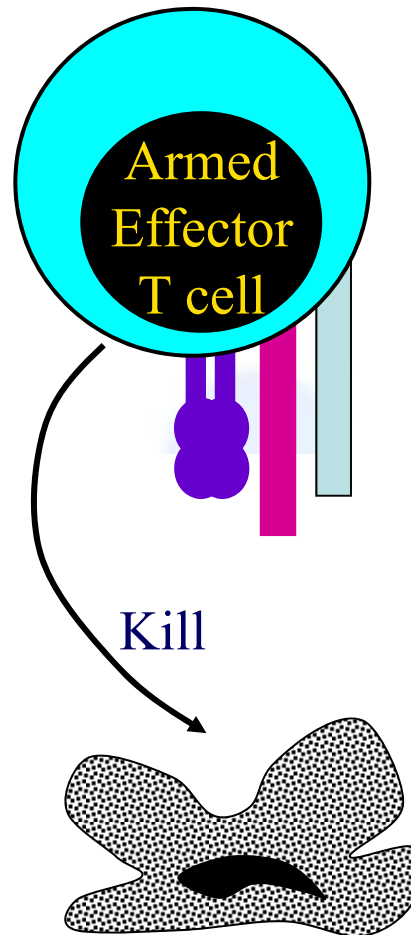
How can this cell give help to, or kill cells, that express low levels of B7 family costimulators?

# Effector function or Anergy?

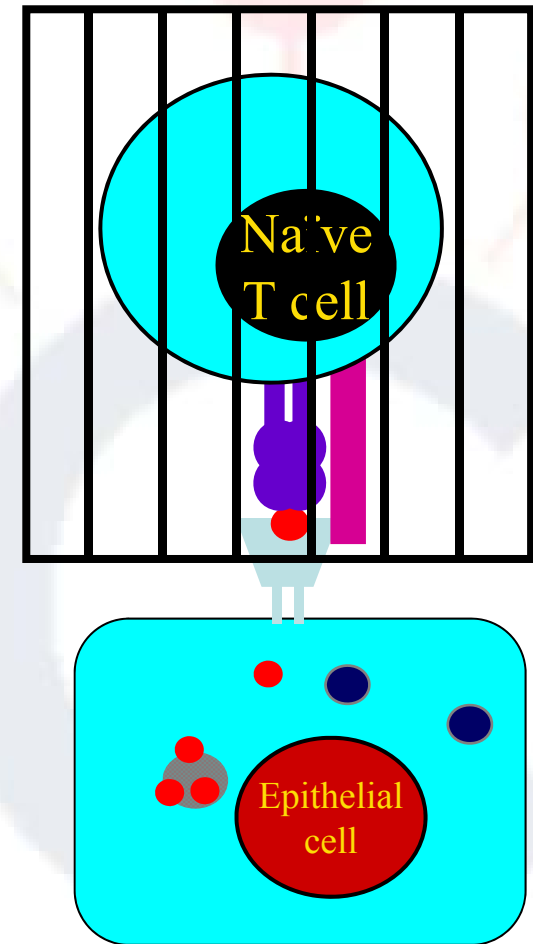
Clonally selected,  
proliferating and  
differentiated  
T cell i.e. **ARMED** sees antigen  
on  
a B7 -ve epithelial cell



The effector programme  
of the T cell is activated  
**without** costimulation



This contrasts the situation  
with naïve T cells, which  
are anergised without  
costimulation

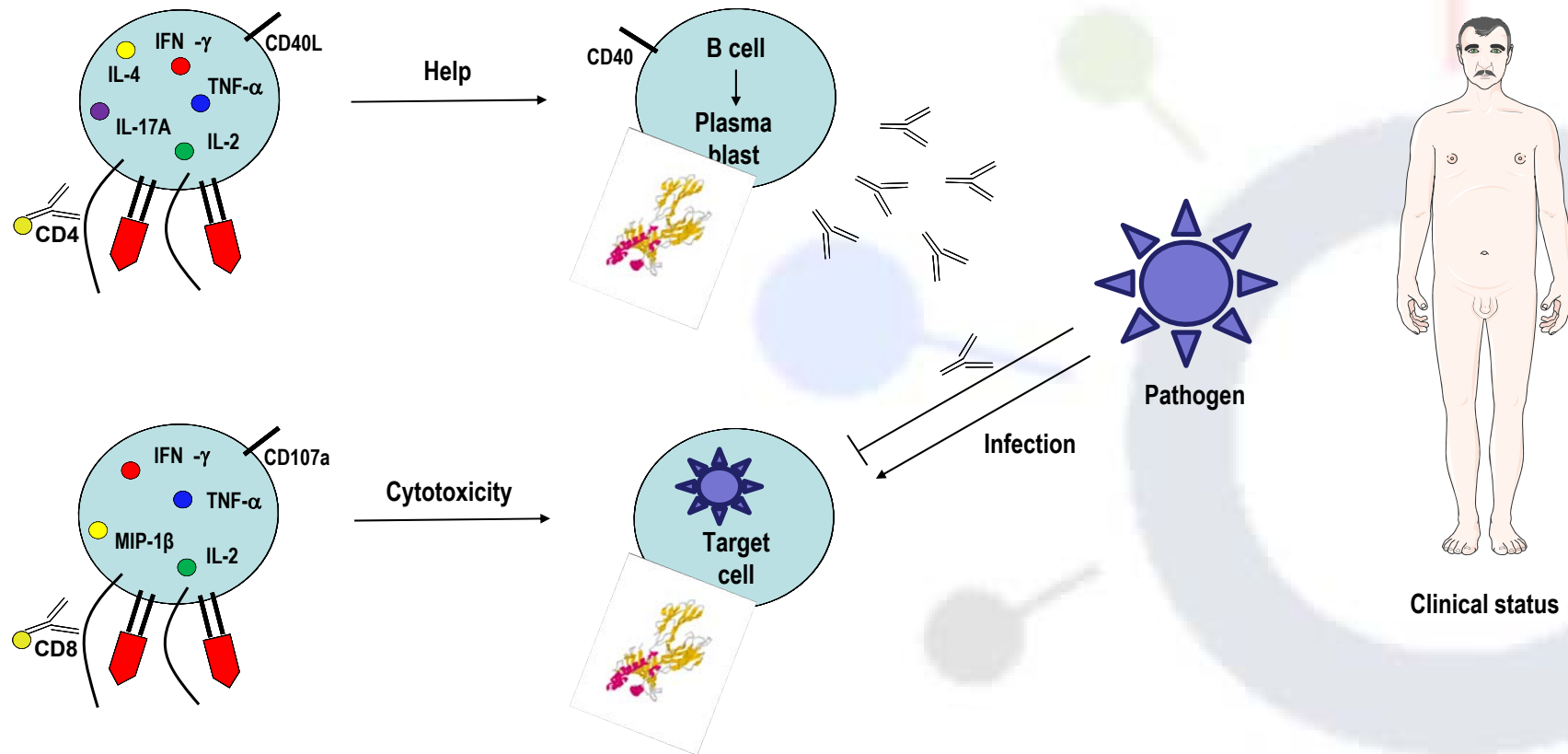




# Protective immunity and T cell efficacy

## Hypothesis:

T cell efficacy *in vitro* and *in vivo* is associated with T cell polyfunctionality.



# Protective immunity and T cell efficacy

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**T cell efficacy *in vitro* and *in vivo* is associated with T cell polyfunctionality.**

## Questions:

- **Does simultaneous expression of effector molecules (polyfunctionality) predict T cell efficacy?**
- **Which T cell derived effector molecules (e.g. cytokines and chemokines) predict T cell efficacy?**

# Protective immunity and T cell efficacy

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## Hypothesis:

T cell efficacy *in vitro* and *in vivo* is associated with T cell polyfunctionality.

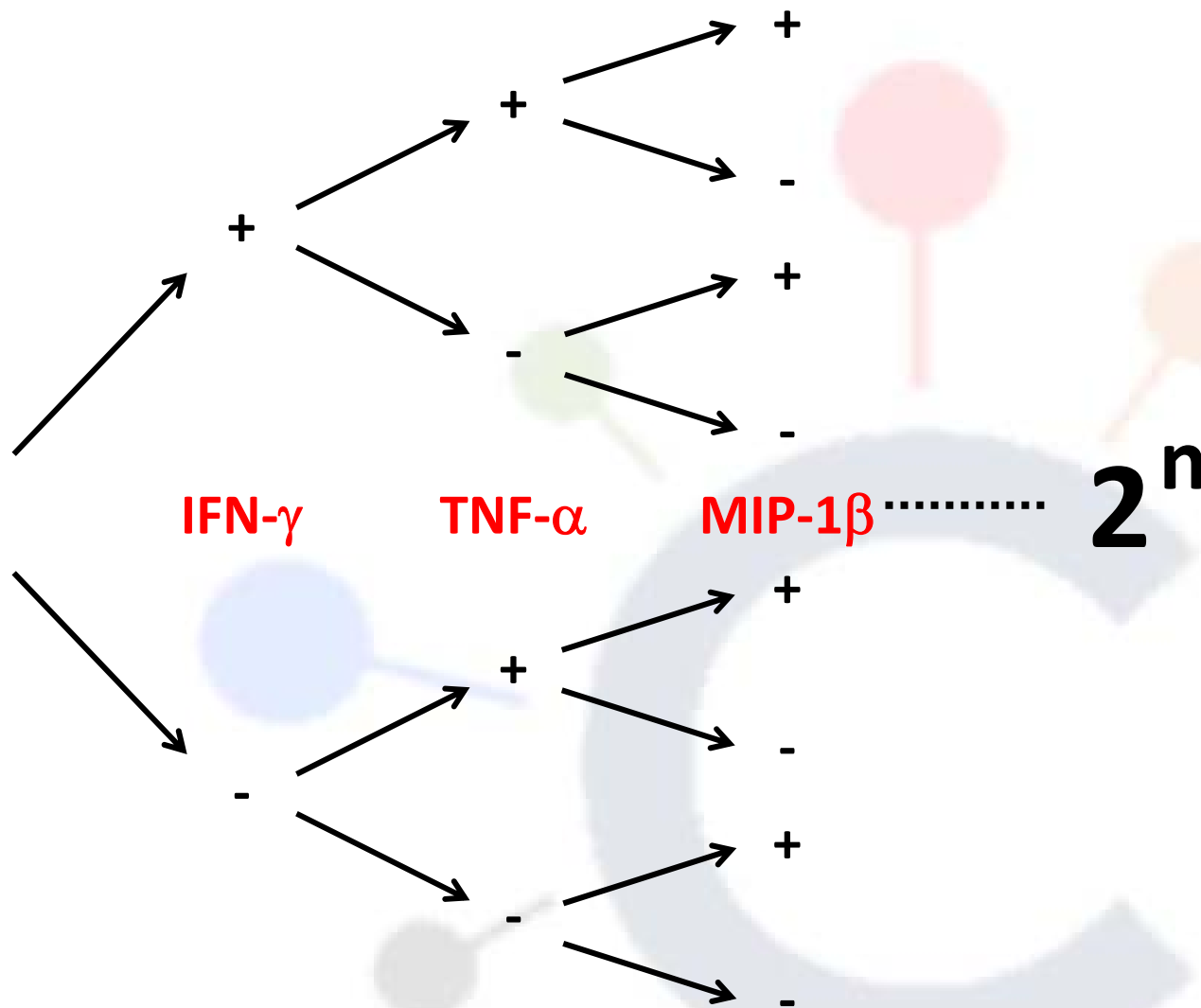
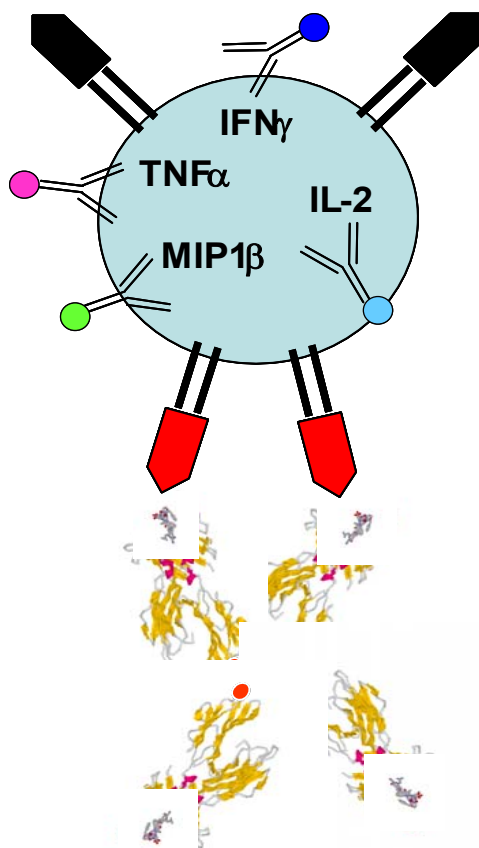
## Questions:

- Does simultaneous expression of effector molecules (polyfunctionality) predict T cell efficacy?
- Which T cell derived effector molecules (e.g. cytokines and chemokines) predict T cell efficacy?

**BUT FIRST AN INTRODUCTION TO  
T CELL POLYFUNCTIONALITY.....**

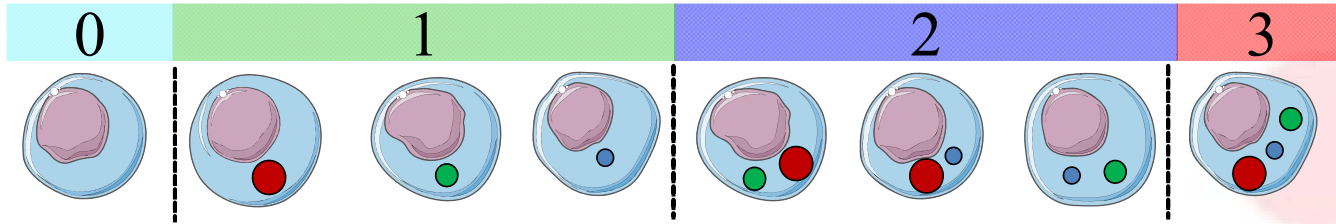
**How to : Measure, analyse and model**

# Exhaustive combinatorial boolean analysis



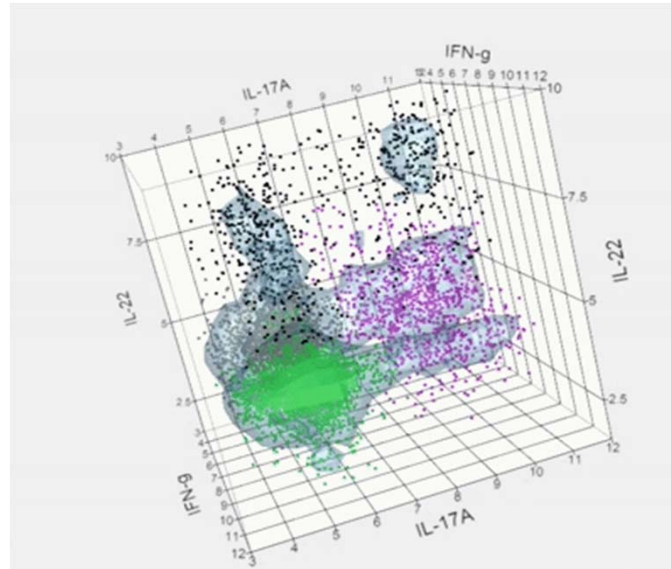
# Exhaustive combinatorial boolean analysis

# of simultaneous functions



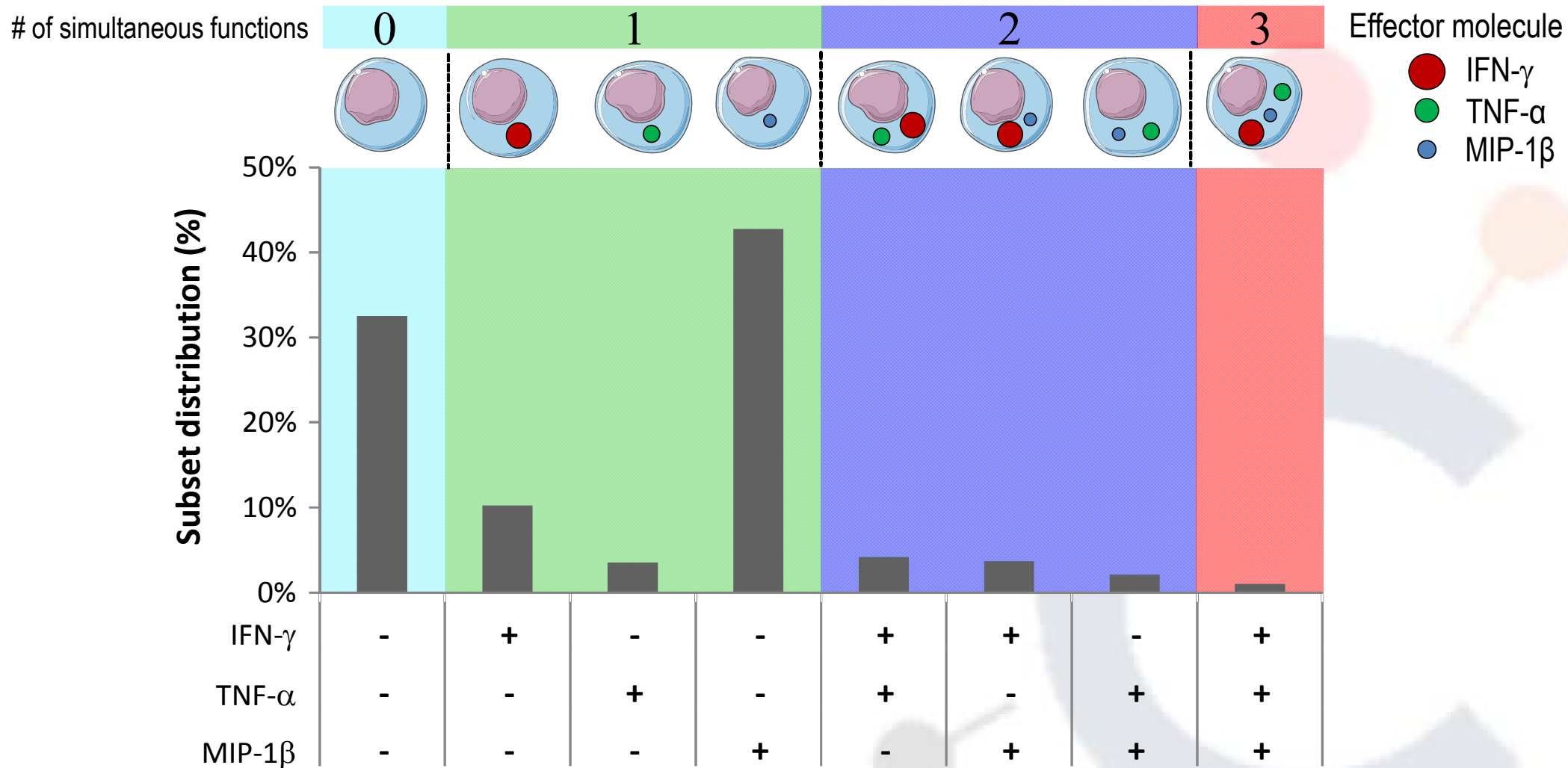
Effector molecule

- IFN- $\gamma$
- TNF- $\alpha$
- MIP-1 $\beta$



IFN- $\gamma$	-	+	-	-	+	+	-	+
TNF- $\alpha$	-	-	+	-	+	-	+	+
MIP-1 $\beta$	-	-	-	+	-	+	+	+

# Exhaustive combinatorial boolean analysis



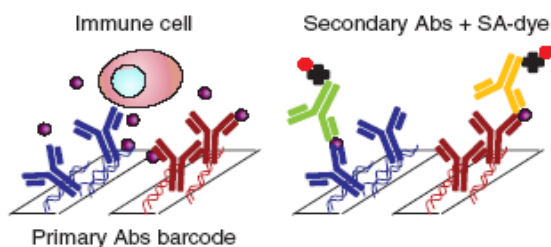
# Exhaustive combinatorial boolean analysis

## TECHNICAL REPORTS

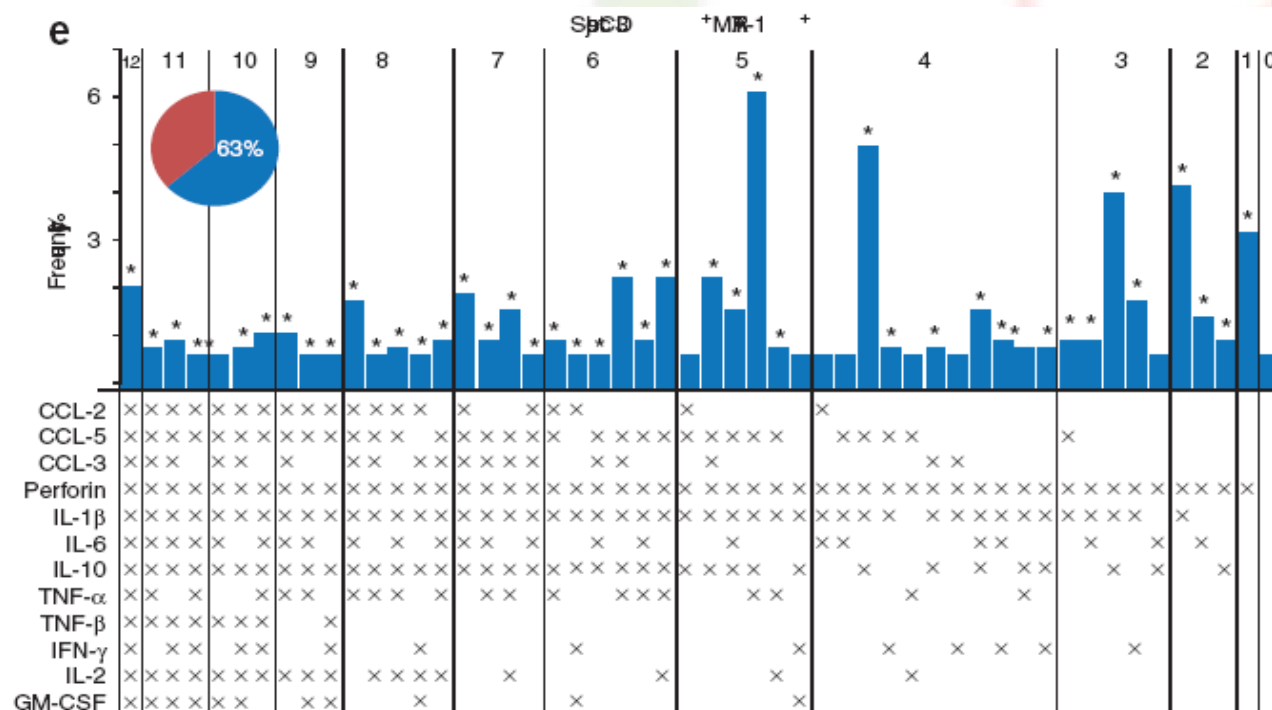
nature  
medicine

A clinical microchip for evaluation of single immune cells reveals high functional heterogeneity in phenotypically similar T cells

Chao Ma<sup>1,2,5</sup>, Rong Fan<sup>1,2,4,5</sup>, Habib Ahmad<sup>1,2</sup>, Qihui Shi<sup>1,2</sup>, Begonya Comin-Anduix<sup>3</sup>, Thinle Chodon<sup>3</sup>, Richard C Koya<sup>3</sup>, Chao-Chao Liu<sup>2</sup>, Gabriel A Kwong<sup>1,2</sup>, Caius G Radu<sup>1,3</sup>, Antoni Ribas<sup>1,3</sup> & James R Heath<sup>1,2</sup>

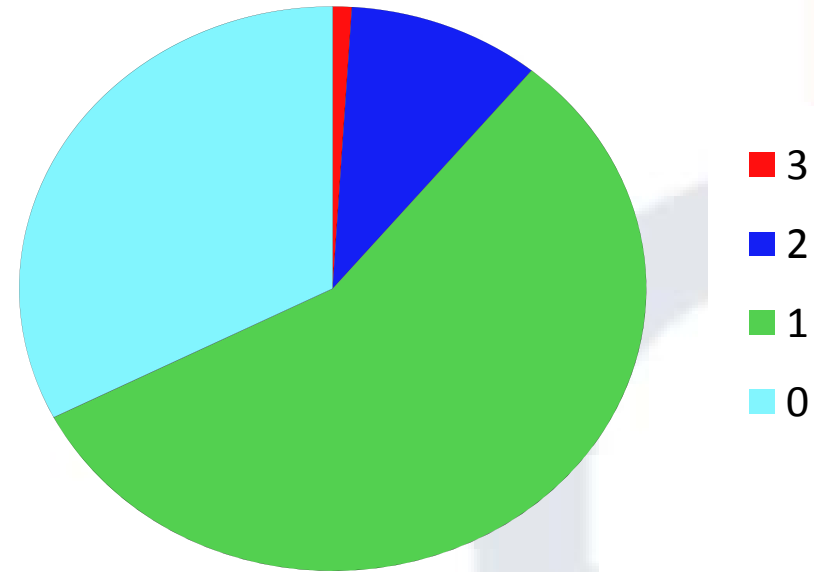
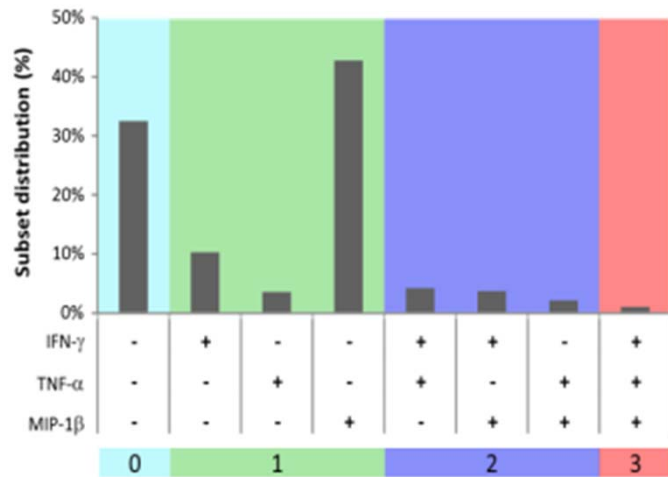


Single-cell  
secretomics.



Ma *et al.* Nat Med 2011

# Qualitative polyfunctionality

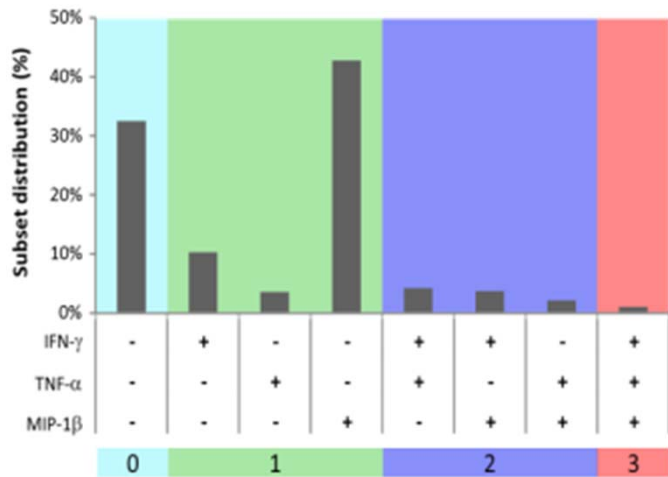




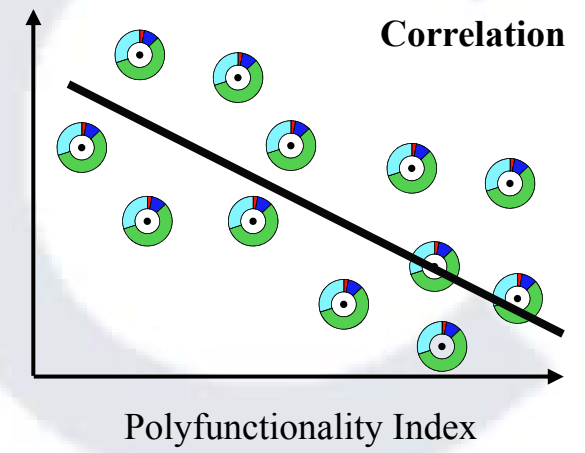
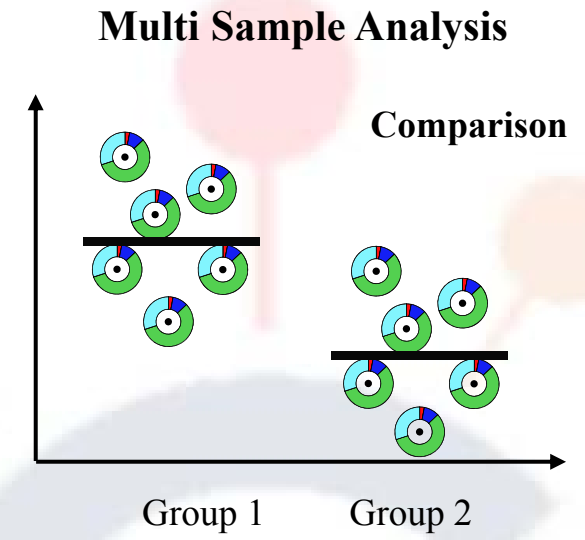
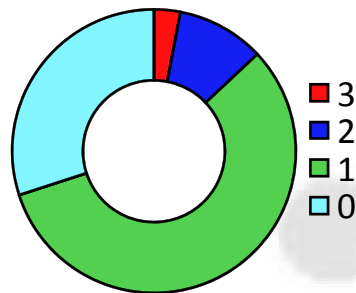
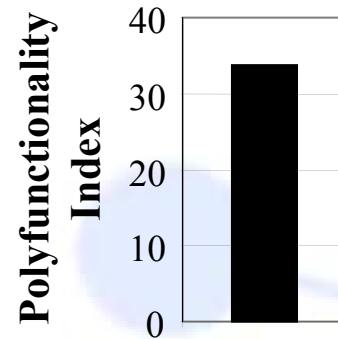
# Quantitative polyfunctionality

## Polyfunctionality Index

$$\frac{\sum_{x_1=0}^1 \sum_{x_2=0}^1 \dots \sum_{x_n=0}^1 (1 + \varphi_{(x_1, x_2, \dots, x_n)}) \cdot F_{\begin{pmatrix} x_1 \\ x_2 \\ \vdots \\ x_n \end{pmatrix}} \cdot \left( \frac{\sum_{i=1}^n x_i}{n} \right)^q}{(1 + \varphi_{(1, \dots, 1)})}$$

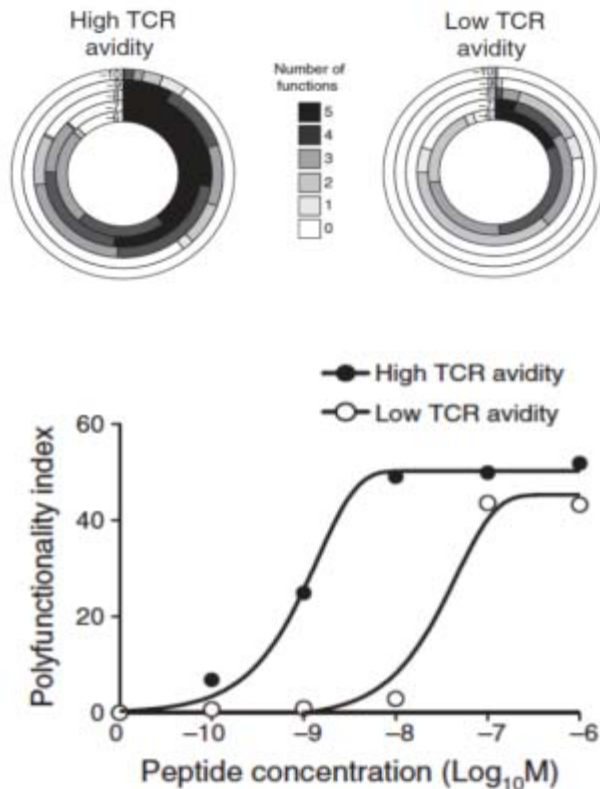


Index  
(1 dimension)



# Applications of the Polyfunctionality Index

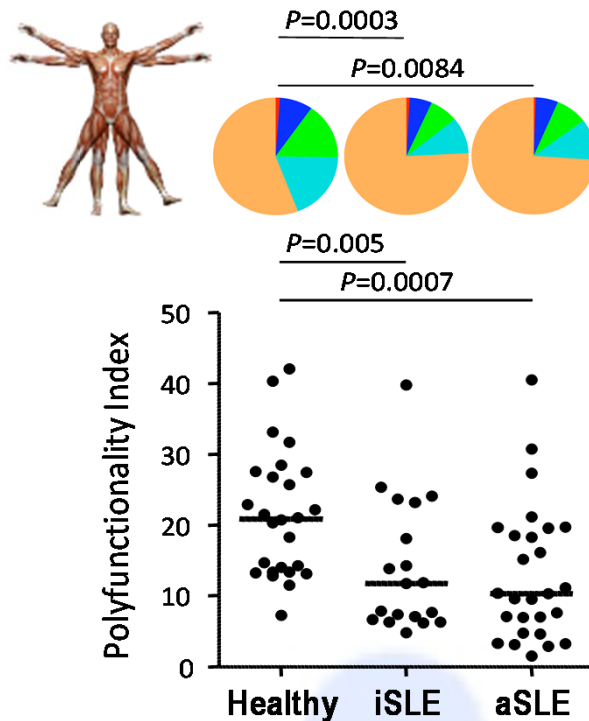
## *In vitro* T cell analysis



- T cell affinity analysis

Lissina *et al.* AIDS 2014  
Hill *et al.* JI 2014  
Lissina *et al.* JI 2016

## *Ex vivo* T cell analysis

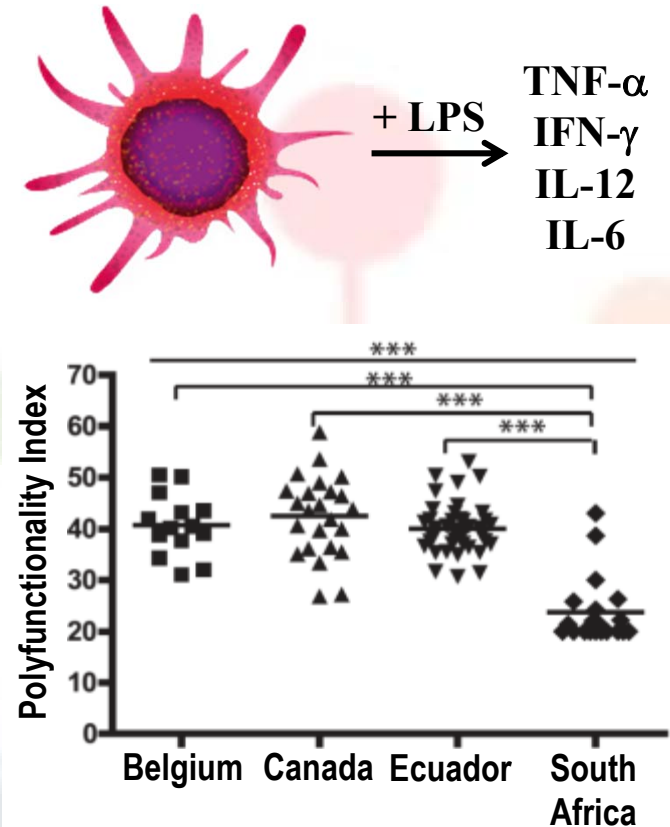


- PD-1<sup>hi</sup> dysfunctional EBV-specific T cells in SLE patients.

Larsen *et al.* PLoS One 2012  
Antoine *et al.* JID 2014  
Goulenok *et al.* AIDS 2014  
Huygens *et al.* JID 2015  
Samri *et al.* AIDS 2016  
Bajwa *et al.* JID 2016  
Lin *et al.* Nat Biotech 2015  
Hassouneh *et al.* Mech Ageing Dev 2016  
Pera *et al.* JLB 2016



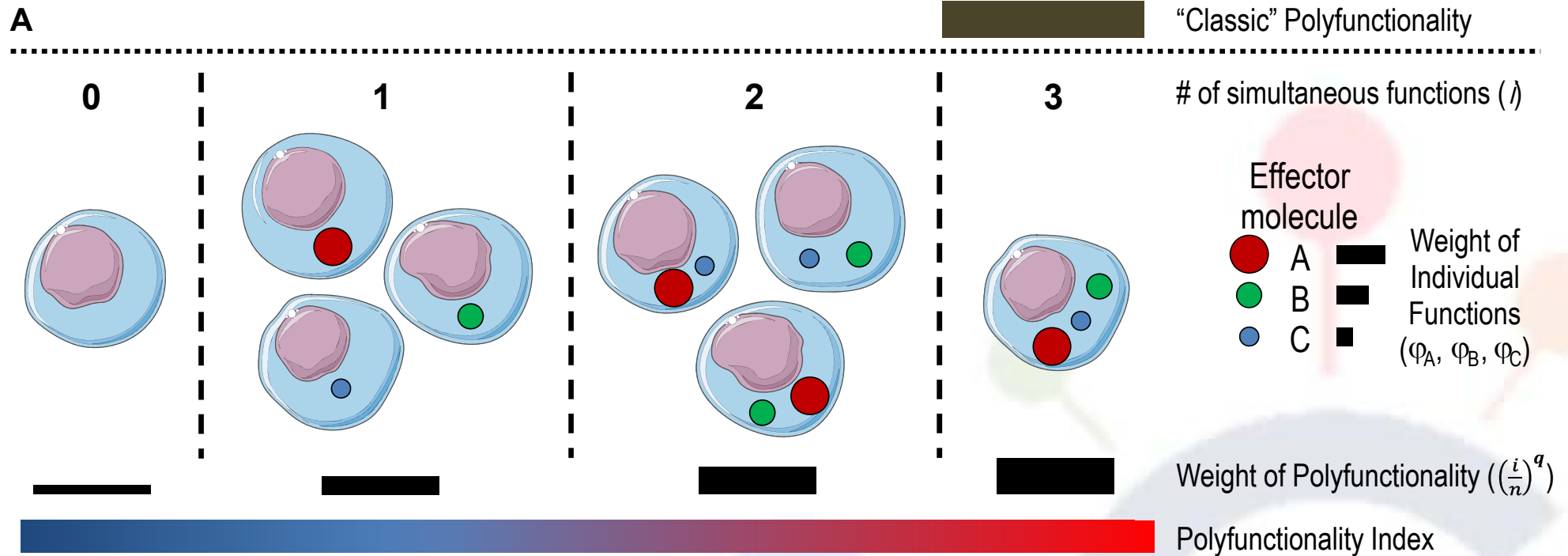
## Dendritic cell analysis



- Dysfunctional DCs from South-African children associated with weak vaccination efficacy.

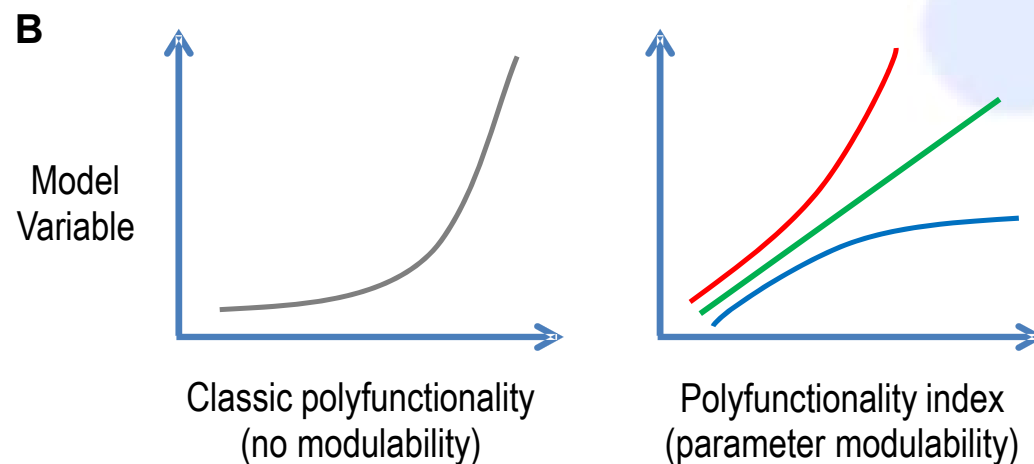
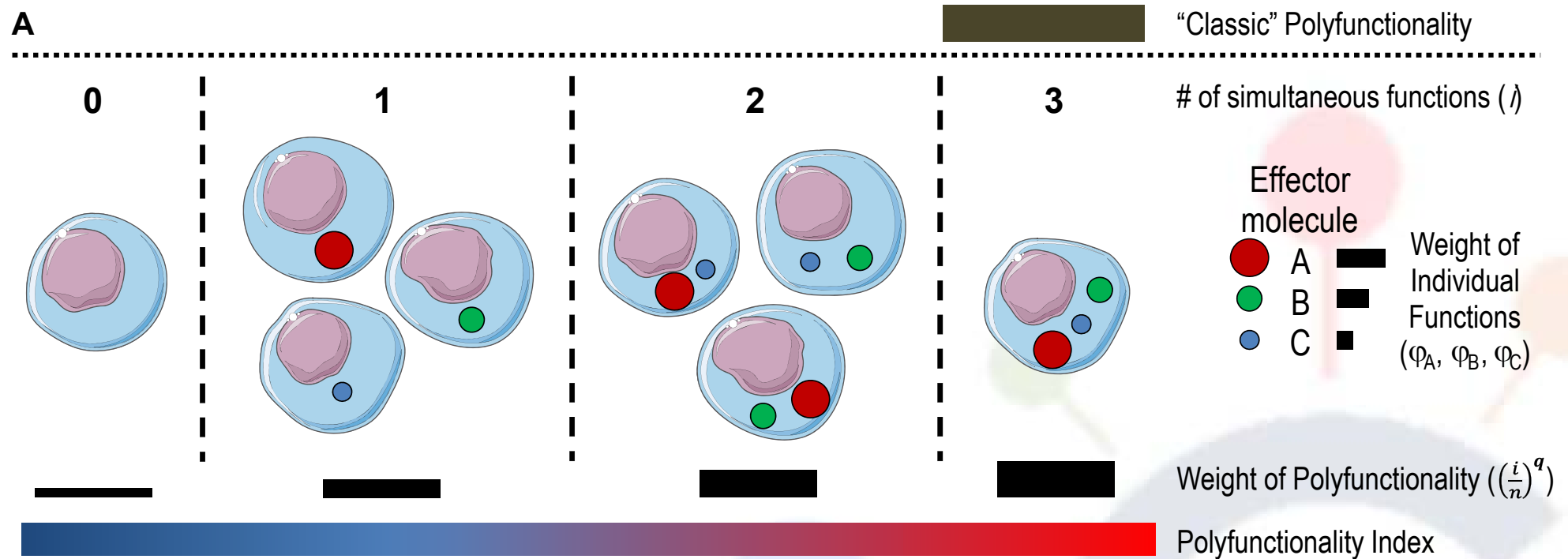
Smolen *et al.* JI 2014

# Parametrisation of the Polyfunctionality Index



$$\frac{\sum_{x_1=0}^1 \sum_{x_2=0}^1 \dots \sum_{x_n=0}^1 (1 + \varphi_{\begin{pmatrix} x_1 \\ x_2 \\ \vdots \\ x_n \end{pmatrix}}) \cdot F_{\begin{pmatrix} x_1 \\ x_2 \\ \vdots \\ x_n \end{pmatrix}} \cdot \left( \frac{\sum_{i=1}^n x_i}{n} \right)^q}{(1 + \varphi_{\begin{pmatrix} 1 \\ 1 \\ \vdots \\ 1 \end{pmatrix}})}$$

# Parametrisation of the Polyfunctionality Index



**C**

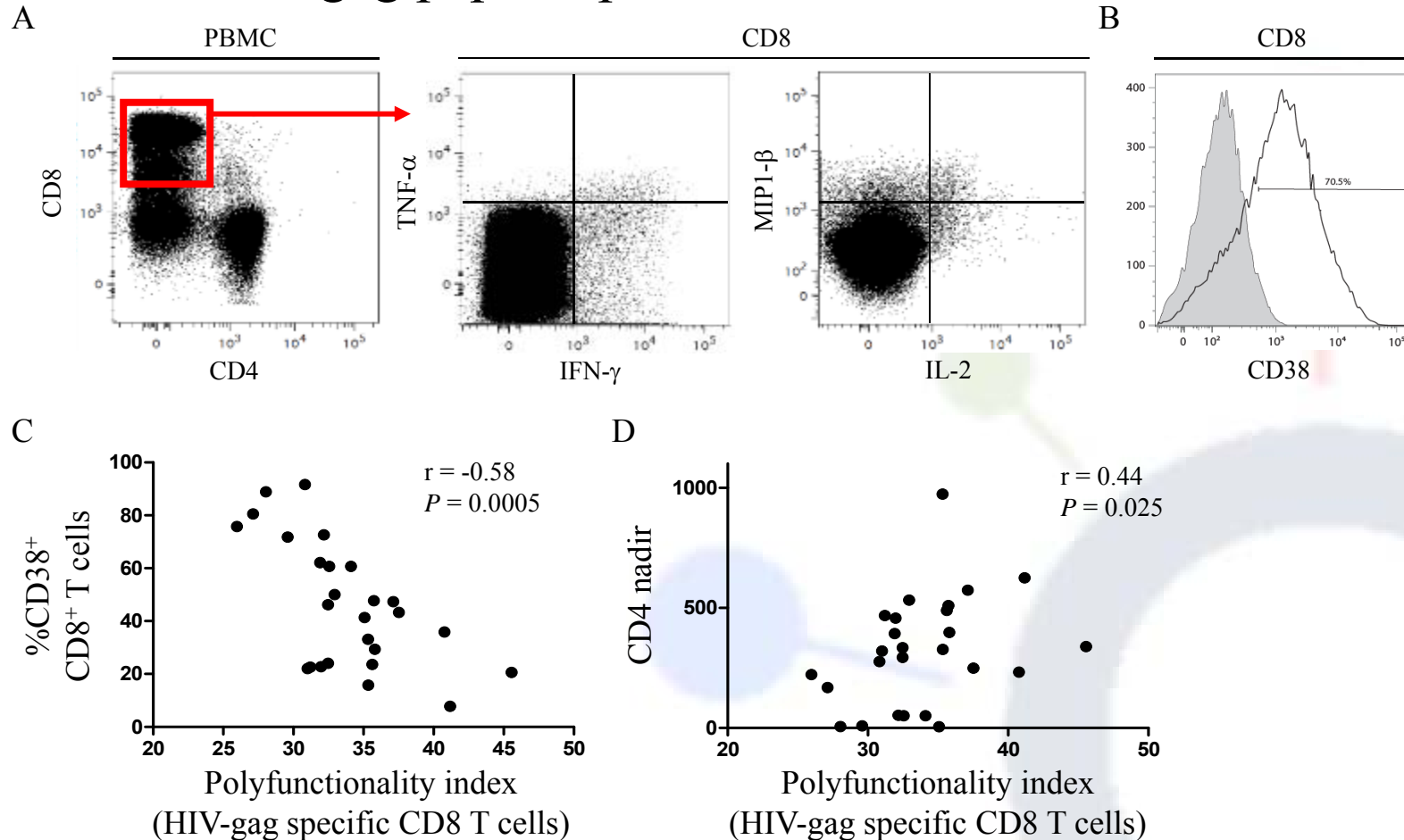
Parameter estimates with inherent biological significance.

$\varphi_A, \varphi_B, \varphi_C$  and  $q$

Boyd *et al.* PLoS One 2015  
 Lin *et al.* Nat Biotech 2015  
 Sauce *et al.* Sci Rep 2016

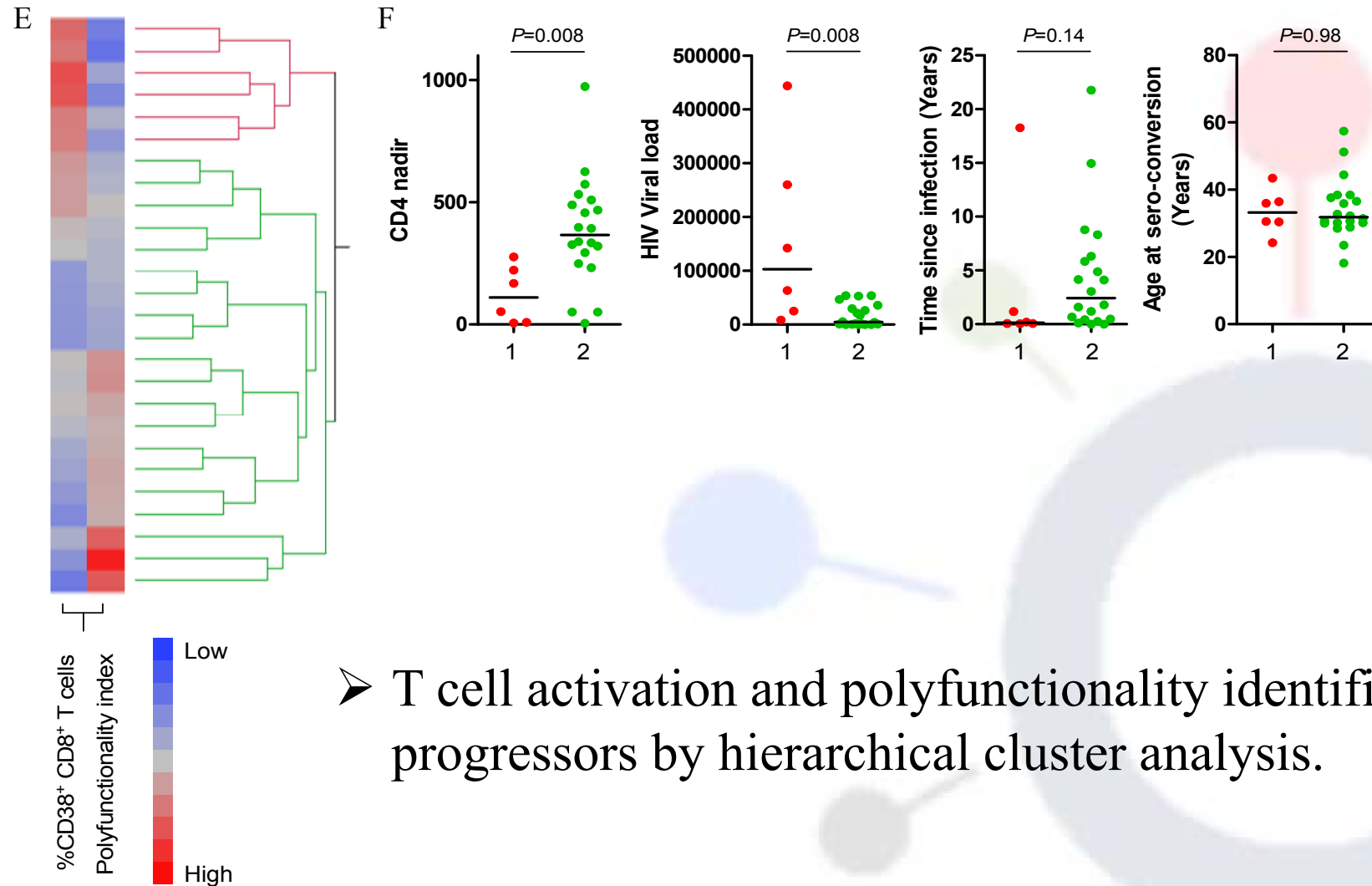
# Polyfunctionality of HIV-gag specific CD8+ T cells

## HIV-gag peptide pool stimulation



- HIV-specific T cell polyfunctionality is negatively correlated with cellular activation (exhaustion) and positively correlated with nadir (lowest CD4<sup>+</sup> T cell count).

# Polyfunctionality of HIV-gag specific CD8+ T cells



➤ T cell activation and polyfunctionality identifies HIV progressors by hierarchical cluster analysis.



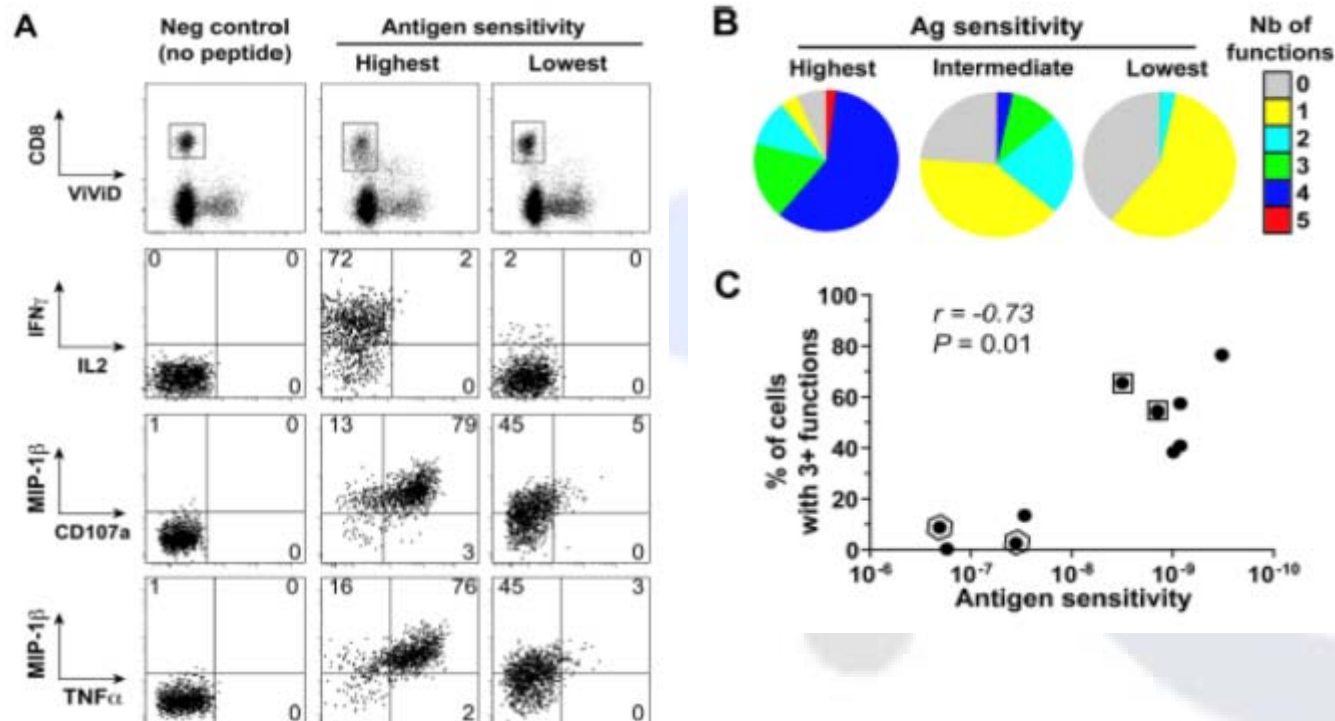
# Polyfunctionality of HIV-gag specific CD8+ T cells

blood

2009 113: 6351-6360  
Prepublished online Apr 23, 2009;  
doi:10.1182/blood-2009-02-206557

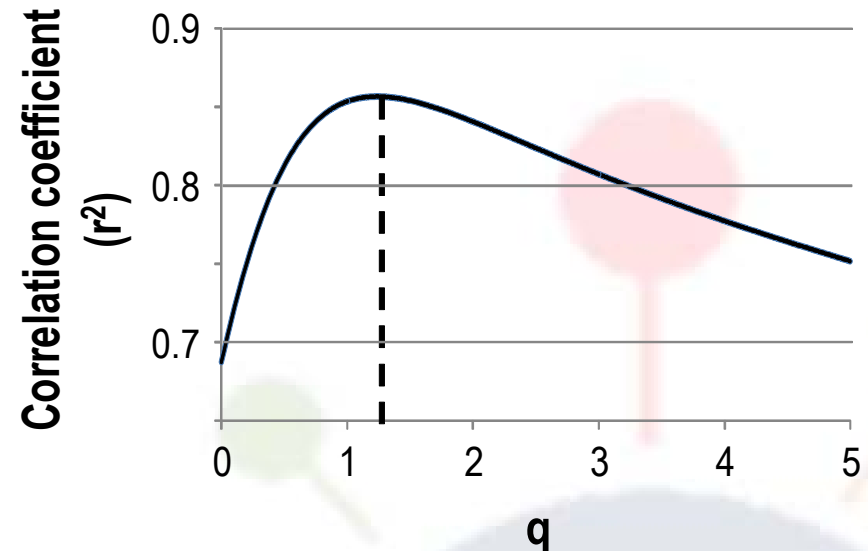
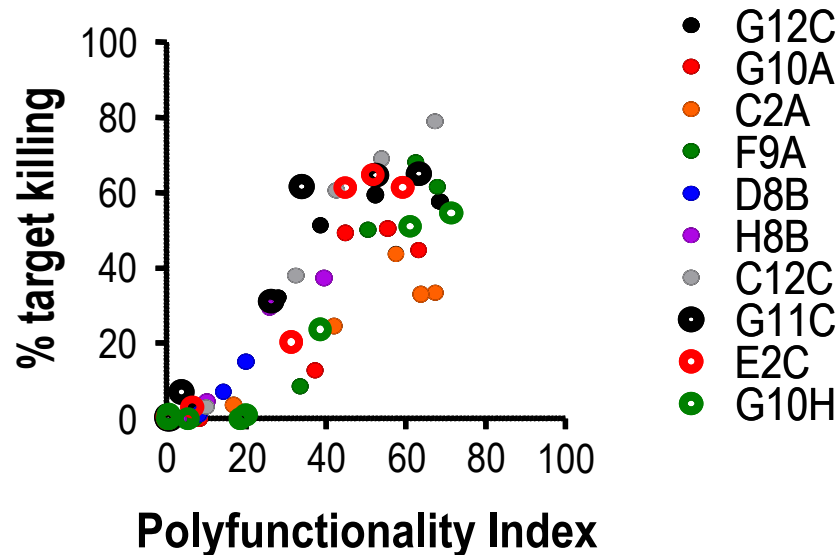
## Antigen sensitivity is a major determinant of CD8<sup>+</sup> T-cell polyfunctionality and HIV-suppressive activity

Jorge R. Almeida,<sup>1</sup> Delphine Sauce,<sup>1</sup> David A. Price,<sup>2,3</sup> Laura Papagno,<sup>1</sup> So Youn Shin,<sup>4</sup> Arnaud Moris,<sup>5</sup> Martin Larsen,<sup>1</sup> Gianfranco Pancino,<sup>4</sup> Daniel C. Douek,<sup>2</sup> Brigitte Autran,<sup>1</sup> Asier Sáez-Cirión,<sup>4</sup> and Victor Appay<sup>1</sup>



Almeida *et al.* Blood 2009

# Polyfunctionality is a correlate of target-killing by HIV-specific T cell clones *in vitro*



**$q=1.2$  is the optimal parameter estimate rendering the polyfunctionality index a predictive measure of target killing.**

## Significance of $q$ :

$q = 0$  : polyfunctionality is not an immune correlate.

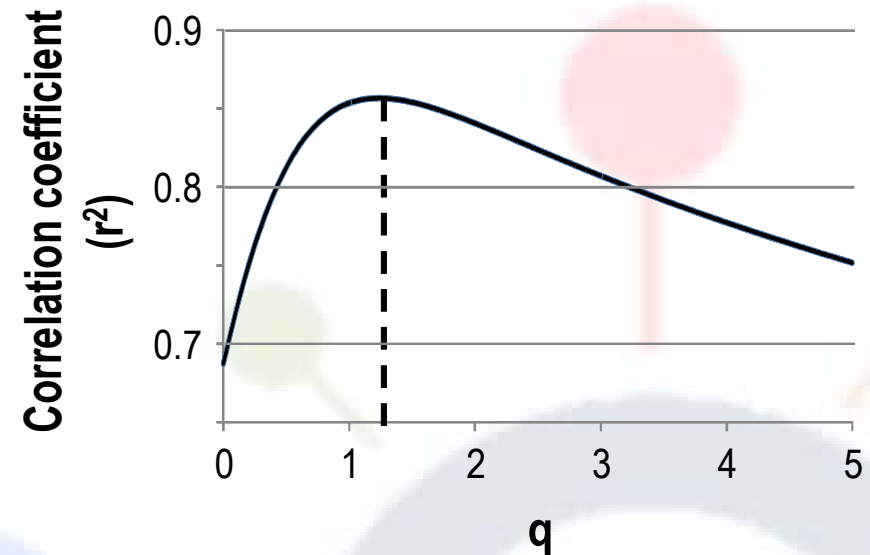
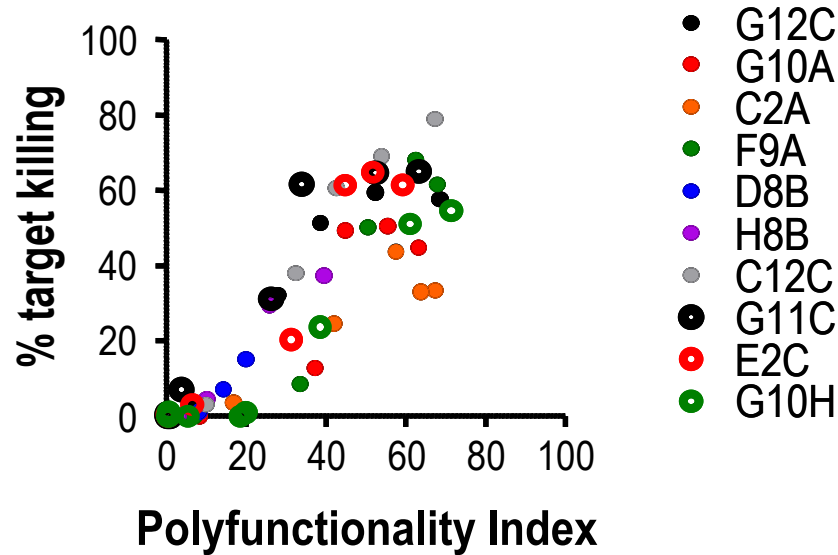
$0 < q < 1$  : polyfunctionality is a moderate immune correlate.

$q > 1$  : polyfunctionality is a strong immune correlate.

$$PI = \sum_{i=0}^n F_i \cdot \left(\frac{i}{n}\right)^q$$



# Polyfunctionality is a correlate of target-killing by HIV-specific T cell clones *in vitro*



$$\frac{\sum_{x_1=0}^1 \sum_{x_2=0}^1 \dots \sum_{x_n=0}^1 (1 + \varphi \begin{pmatrix} x_1 \\ x_2 \\ \vdots \\ x_n \end{pmatrix}) \cdot F \begin{pmatrix} x_1 \\ x_2 \\ \vdots \\ x_n \end{pmatrix} \cdot \left( \frac{\sum_{i=1}^n x_i}{n} \right)^q}{(1 + \varphi \begin{pmatrix} 1 \\ \vdots \\ 1 \end{pmatrix})}$$

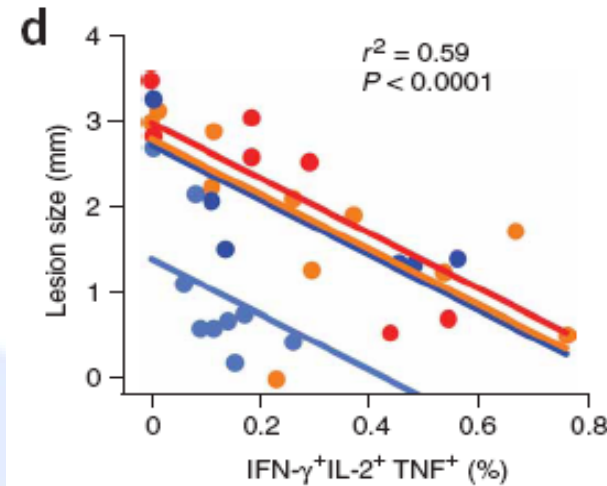
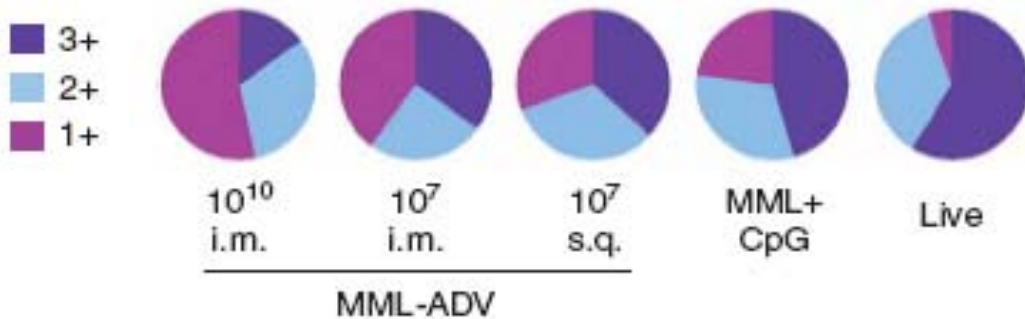
Parameters	Model 1		Model 2	
	Estimate (95% CI)	<i>p</i>	Estimate (95% CI)	<i>p</i>
<i>q</i>	1.24 (0.85, 1.63)	<b>&lt;0.001</b>	3.05 (1.30, 4.81)	<b>0.001</b>
$\phi$				
CD107a	--		-0.96 (-3.96, 2.03)	0.5
TNF- $\alpha$	--		-5.65 (-12.38, 1.08)	0.10
IFN- $\gamma$	--		1.04 (-0.36, 2.44)	0.14
MIP-1 $\beta$	--		5.86 (-4.32, 16.03)	0.3
IL-2	--		-0.62 (-1.62, 0.39)	0.2
Correlation (R <sup>2</sup> )	0.8563		0.9144	

# Polyfunctionality is a correlate of *Leishmania major* vaccine efficacy *in vivo*

nature  
medicine

Multifunctional T<sub>H</sub>1 cells define a correlate of vaccine-mediated protection against *Leishmania major*

Patricia A Darrah<sup>1</sup>, Dipti T Patel<sup>1</sup>, Paula M De Luca<sup>1</sup>, Ross W B Lindsay<sup>1</sup>, Dylan F Davey<sup>1</sup>, Barbara J Flynn<sup>1</sup>, Søren T Hoff<sup>2</sup>, Peter Andersen<sup>2</sup>, Steven G Reed<sup>3</sup>, Sheldon L Morris<sup>4</sup>, Mario Roederer<sup>5</sup> & Robert A Seder<sup>1</sup>

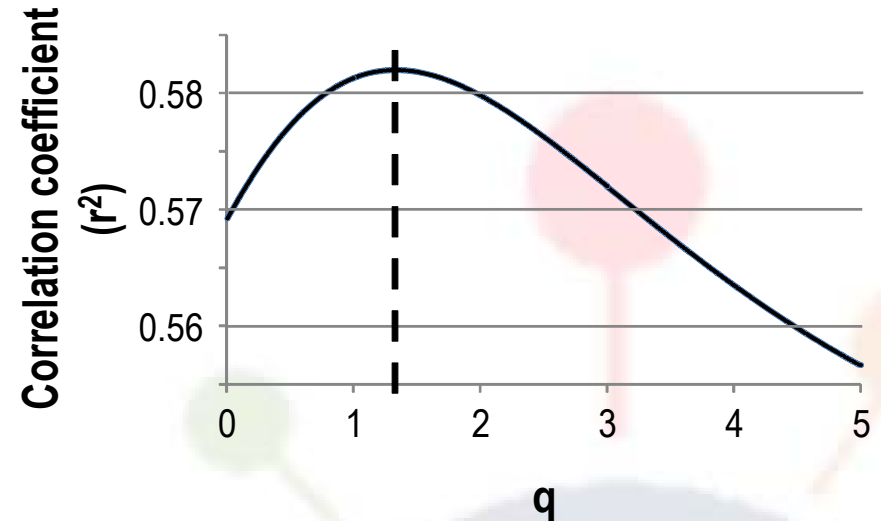
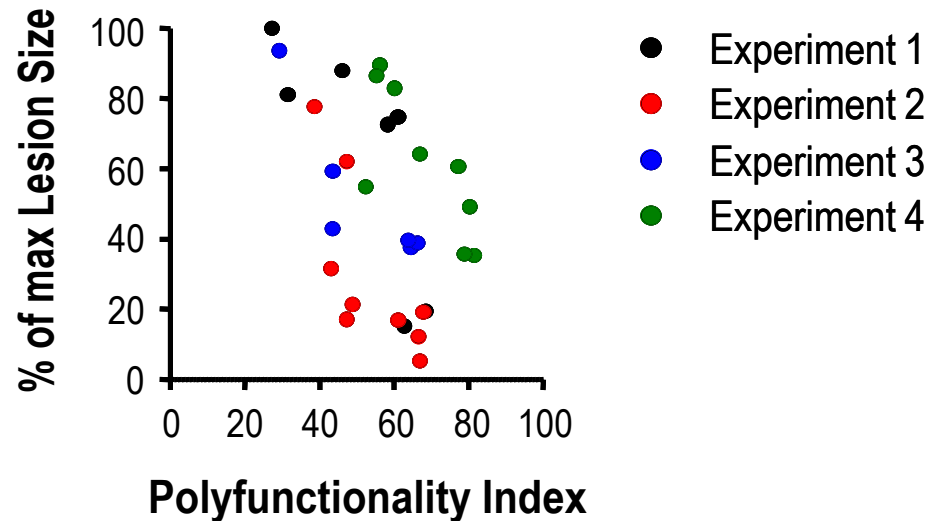


Corresponds to special PI with  $q = \infty$

$$\text{Special Polyfunctionality Index} = \sum_{i=0}^n F_i \cdot \left(\frac{i}{n}\right)^q \xrightarrow{q=\infty} F_n$$

Darrah *et al.* Nat Med 2007

# Polyfunctionality is a correlate of *Leishmania major* vaccine efficacy *in vivo*



**$q=1.3$  is the optimal parameter estimate rendering the polyfunctionality index a predictive measure of vaccine efficacy.**

## Significance of $q$ :

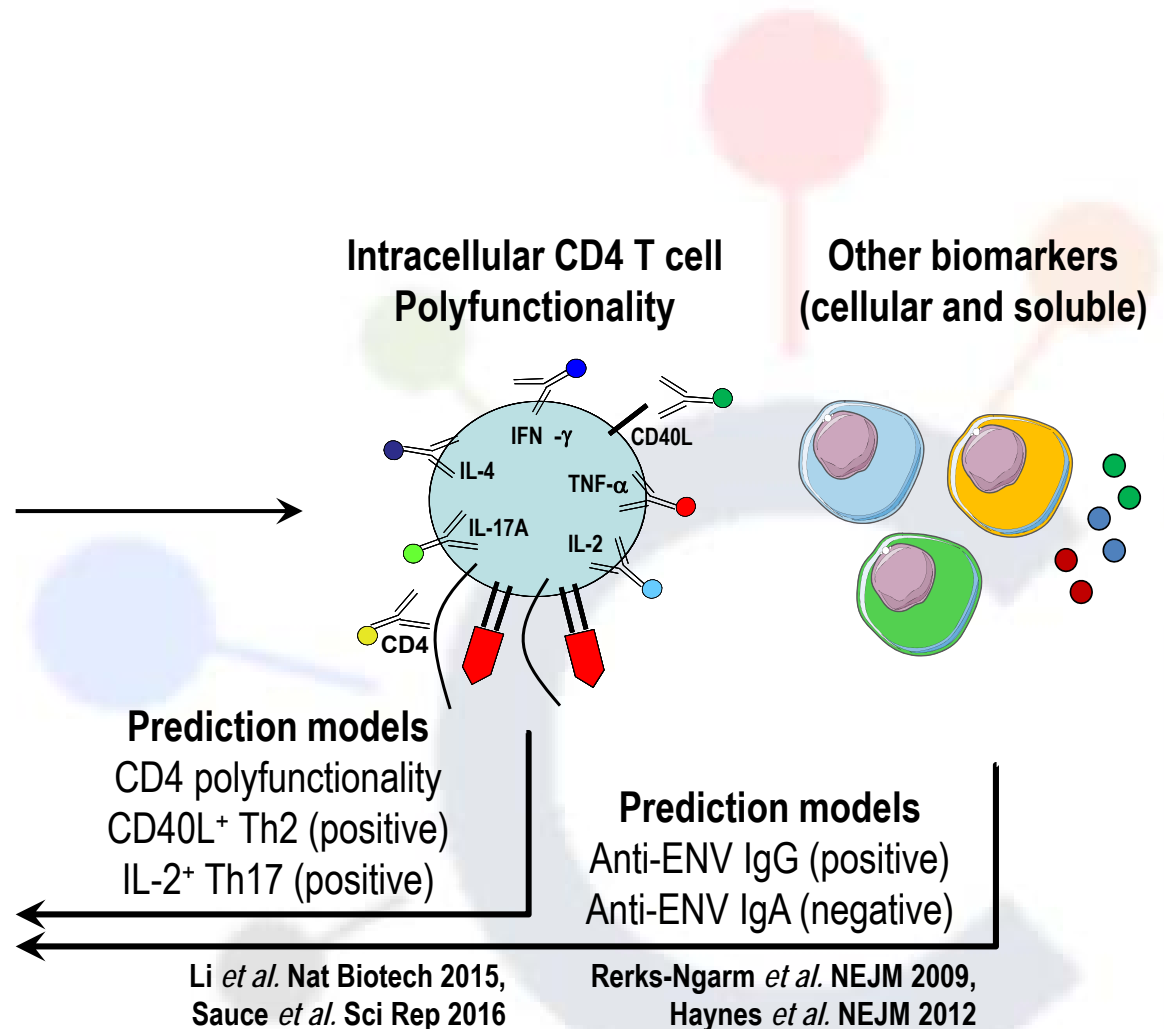
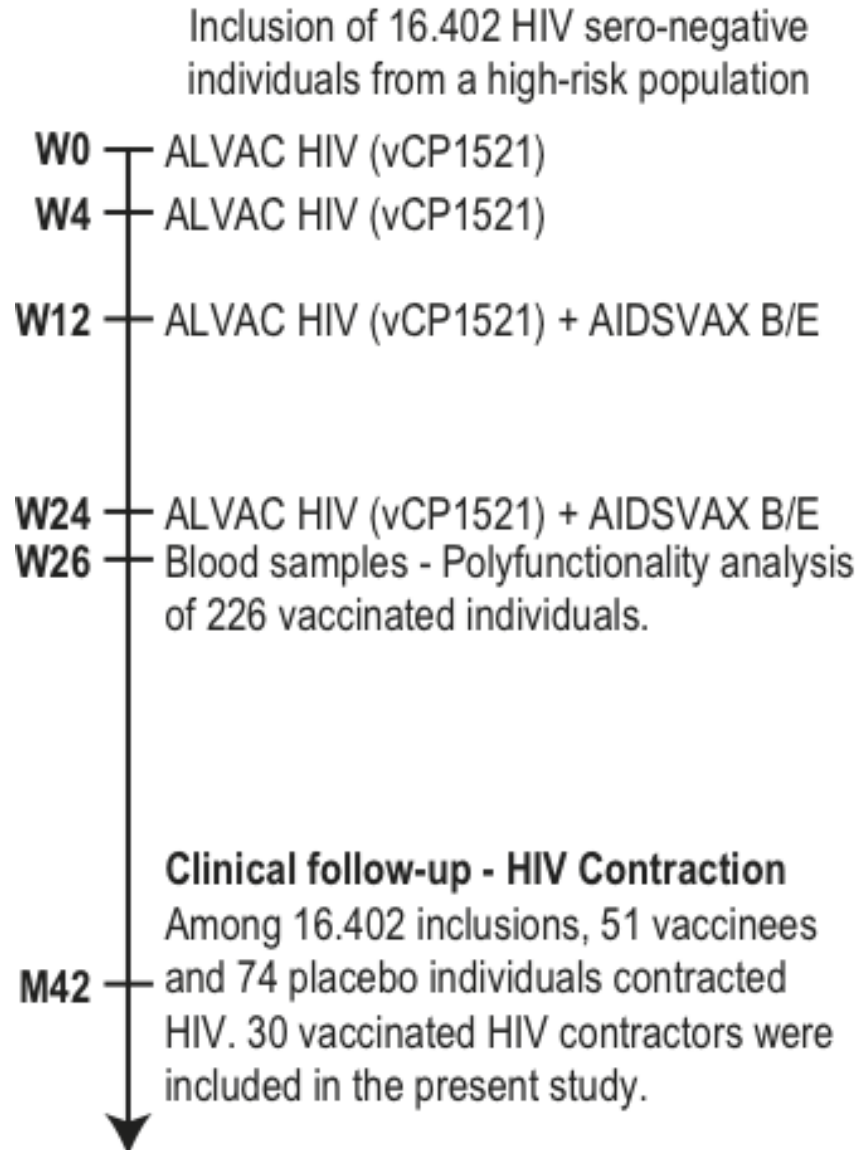
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# RV144 trial design and output

## RV144 HIV case-control clinical trial:



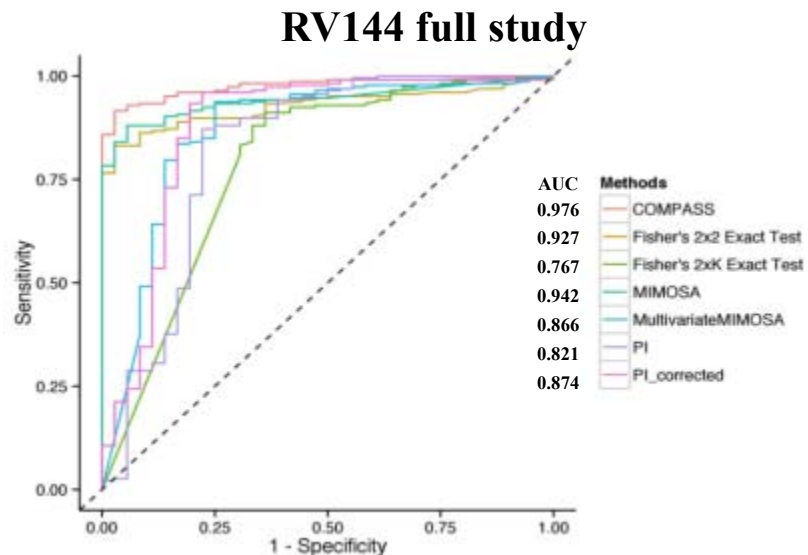
# COMPASS - a Bayesian hierarchical framework model

## RV144 HIV case-control clinical trial:

**Vaccine:** ALVAC HIV (vCP1521) + AIDSVAX B/E (gp120)

**Inclusion:** 125 contracted HIV (cases) of 16,402 HIV seronegative participants.

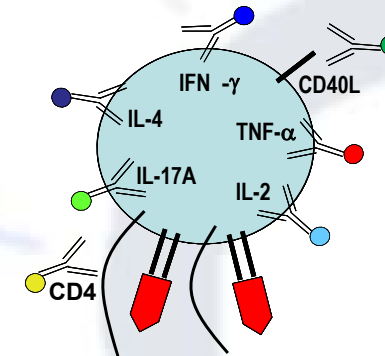
**Protection:** Infection: 51 vaccinees and 74 placebo. 31% reduction in HIV infection over 3 years follow-up.



**Prediction:** Vaccinee vs placebo

**Inclusion:** 226 vaccinees and 40 placebo

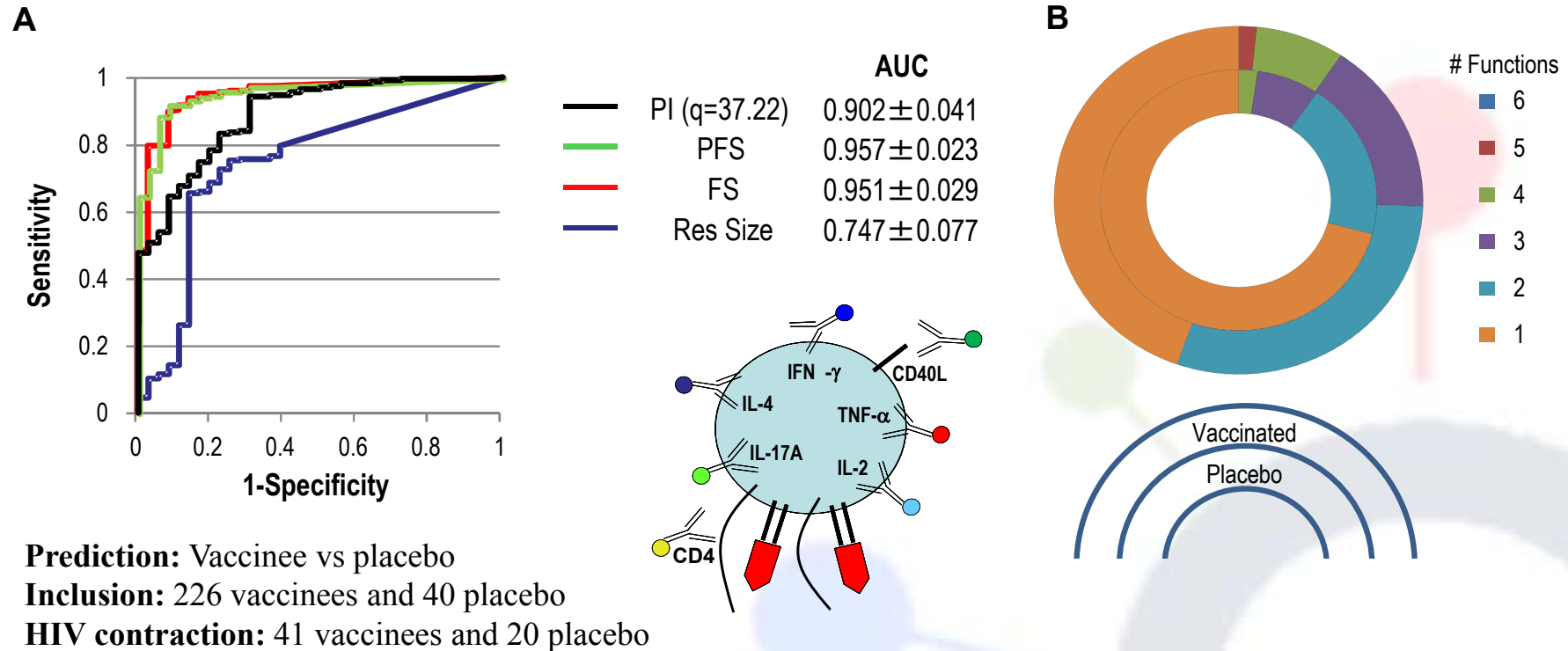
**HIV contraction:** 41 vaccinees and 20 placebo



Lin *et al.* Nature Biotechnology 2015

# COMPASS - a Bayesian hierarchical framework model

## RV144 HIV case-control clinical trial:



**T cell quantity = Response size (applicable to samples with and without HIV-specific T cells).**

**T cell quality = Polyfunctionality (applicable only to samples with HIV-specific T cells).**

**Why is response size a less good predictor compared to polyfunctionality?**



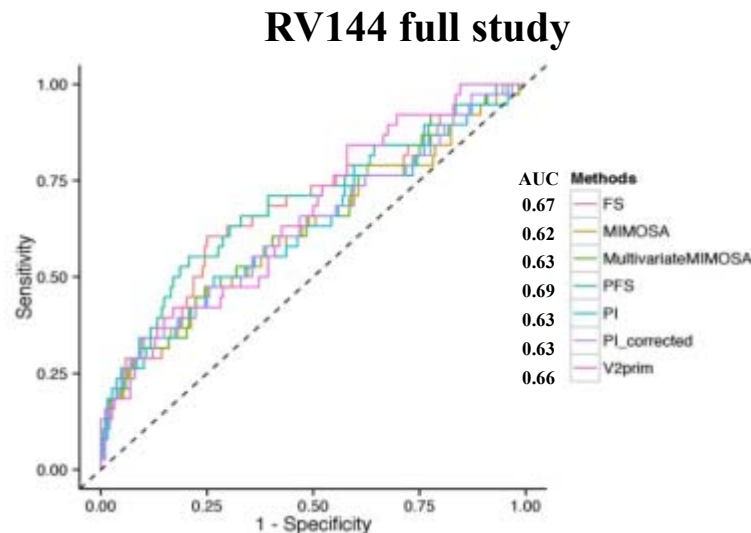
# COMPASS - a Bayesian hierarchical framework model

## RV144 HIV case-control clinical trial:

**Vaccine:** ALVAC HIV (vCP1521) + AIDSVAX B/E (gp120)

**Inclusion:** 125 contracted HIV (cases) of 16,402 HIV seronegative participants.

**Protection:** Infection: 51 vaccinees and 74 placebo. 31% reduction in HIV infection over 3 years follow-up.



**Prediction:** HIV contraction

**Inclusion:** 226 vaccinees and 40 placebo

**HIV contraction:** 41 vaccinees and 20 placebo

### Bayesian probability (poly)functionality scores:

**Table 1** Estimated odds ratios for HIV-1 infection risk for the subset specific responses as determined by logistic regression models that adjust for baseline risk category and gender in the RV144 case-control study

Variable	Odds ratio (95% CI)	P-value	q-value
FS	0.62 (0.42, 0.91)	0.014	0.06
PFS	0.57 (0.38, 0.84)	0.005	0.05
IL-4+ IL-2+ CD40L+	0.62 (0.43, 0.90)	0.013	0.06
TNF- $\alpha$ + IFN- $\gamma$ + IL-4+ IL-2+ CD40L+	0.58 (0.39, 0.86)	0.006	0.05

Only subsets with  $P < 0.05$  are shown here. Odds ratio are per one s.d. for each variable and are adjusted for IgA level, gender and baseline behavioral risk score. Lower and upper limits of the 95% confidence intervals (CI) for the estimated ratios are also shown. Q-values are the FDR-adjusted P-values across all 17 considered variables.

### Frequency adjusted (poly)functionality scores:

Variable	Odds ratio (95% CI)	p-value	q-value
Functionality score (P)	1.094(0.80 – 1.51)	0.584	0.959
Polyfunctionality score (P)	1.075(0.78 – 1.49)	0.663	0.959
IL4+IL2+CD40L+ (P)	0.926(0.66 – 1.30)	0.656	0.959
TNF $\alpha$ +IFN $\gamma$ +IL4+IL2+CD40L+ (P)	0.946(0.66 – 1.35)	0.761	0.959

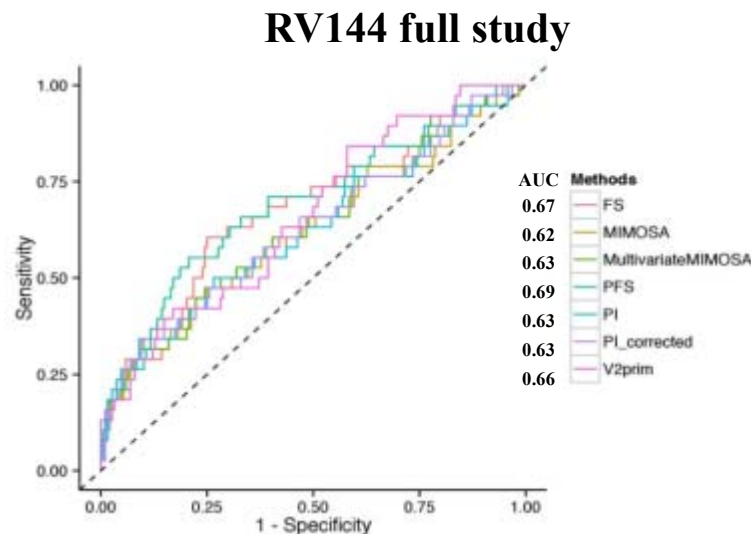
# COMPASS - a Bayesian hierarchical framework model

## RV144 HIV case-control clinical trial:

**Vaccine:** ALVAC HIV (vCP1521) + AIDSVAX B/E (gp120)

**Inclusion:** 125 contracted HIV (cases) of 16,402 HIV seronegative participants.

**Protection:** Infection: 51 vaccinees and 74 placebo. 31% reduction in HIV infection over 3 years follow-up.



**Prediction:** HIV contraction

**Inclusion:** 226 vaccinees and 40 placebo

**HIV contraction:** 41 vaccinees and 20 placebo

**1/3 of non-controllers are placebo versus 1/10 in controllers. HUGE BIAS....**

### Bayesian probability (poly)functionality scores:

**Table 1** Estimated odds ratios for HIV-1 infection risk for the subset specific responses as determined by logistic regression models that adjust for baseline risk category and gender in the RV144 case-control study

Variable	Odds ratio (95% CI)	P-value	q-value
FS	0.62 (0.42, 0.91)	0.014	0.06
PFS	0.57 (0.38, 0.84)	0.005	0.05
IL-4 <sup>+</sup> IL-2 <sup>+</sup> CD40L <sup>+</sup>	0.62 (0.43, 0.90)	0.013	0.06
TNF- $\alpha$ <sup>+</sup> IFN- $\gamma$ <sup>+</sup> IL-4 <sup>+</sup> IL-2 <sup>+</sup> CD40L <sup>+</sup>	0.58 (0.39, 0.86)	0.006	0.05

Only subsets with  $P < 0.05$  are shown here. Odds ratio are per one s.d. for each variable and are adjusted for IgA level, gender and baseline behavioral risk score. Lower and upper limits of the 95% confidence intervals (CI) for the estimated ratios are also shown. Q-values are the FDR-adjusted P-values across all 17 considered variables.

### Alternative (poly)functionality scores:

Variable	Odds ratio (95% CI)	p-value
MultivariateMIMOSA	0.884(0.62 – 1.27)	0.501
MIMOSA	0.882(0.62 – 1.26)	0.495
Polyfunctionality Index	1.083(0.77 – 1.52)	0.641
Polyfunctionality Index (corrected)	1.153(0.84 – 1.58)	0.376
Number of functions by MBA	0.581(0.38 – 0.89)	0.013

Lin *et al.* Nature Biotechnology 2015



# CD4<sup>+</sup> T cell polyfunctionality and HIV vaccine protection

## **RV144 HIV case-control clinical trial:**

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Rerks-Ngarm *et al.* NEJM 2009, Haynes *et al.* NEJM 2012, Li *et al.* Nat Biotech 2015

## **Model adjustment:**

Removal of placebo treated individuals and non-responders severely reduces the predictive capacity of polyfunctionality.

## **Our study design:**

**Prediction:** HIV contraction

**Inclusion:** 185 vaccinees with detectable HIV-specific T cells.

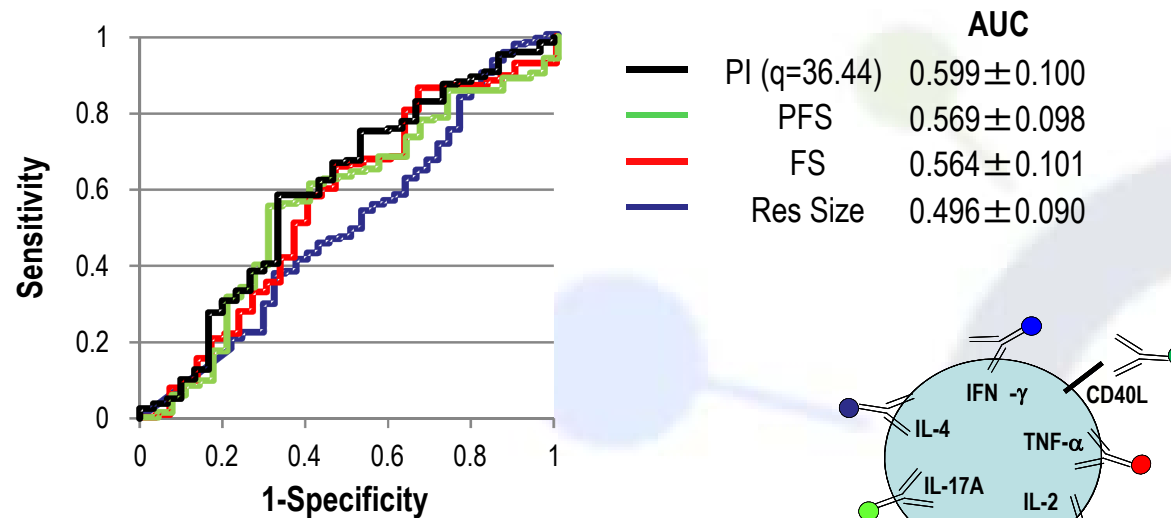
**HIV contraction:** 30 out of 185 vaccinees included in the analysis

# COMPASS versus Polyfunctionality after adjustment

## Predictive model of HIV contraction:

*Ex vivo* polyfunctional analysis of HIV-gag specific CD4<sup>+</sup> T cells post vaccination (IFN- $\gamma$ , IL-4, IL-17A, CD40L, IL-2, TNF- $\alpha$ ).

**Independent variables:** Polyfunctionality Index (PI), (Poly)Functionality Score (PFS, FS) and Response Size (Res Size)



**Prediction:** HIV contraction

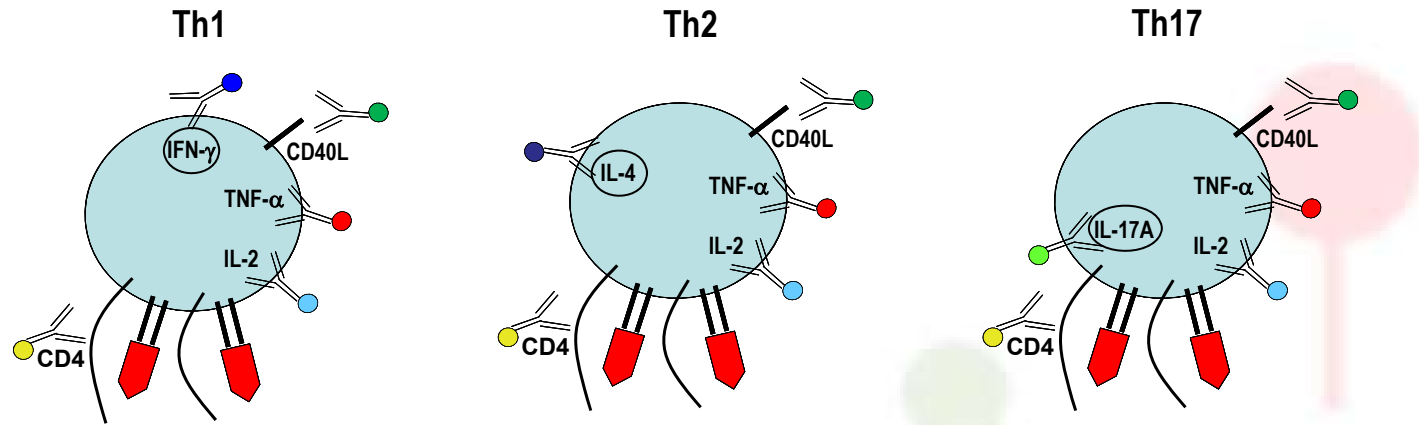
**Inclusion:** 185 vaccinees with detectable HIV-specific T cells.

**HIV contraction:** 30 vaccinees

Lin *et al.* Nat Biotech 2015, Sauce *et al.* Sci Rep 2016

# CD4<sup>+</sup> T cell polyfunctionality and HIV vaccine protection

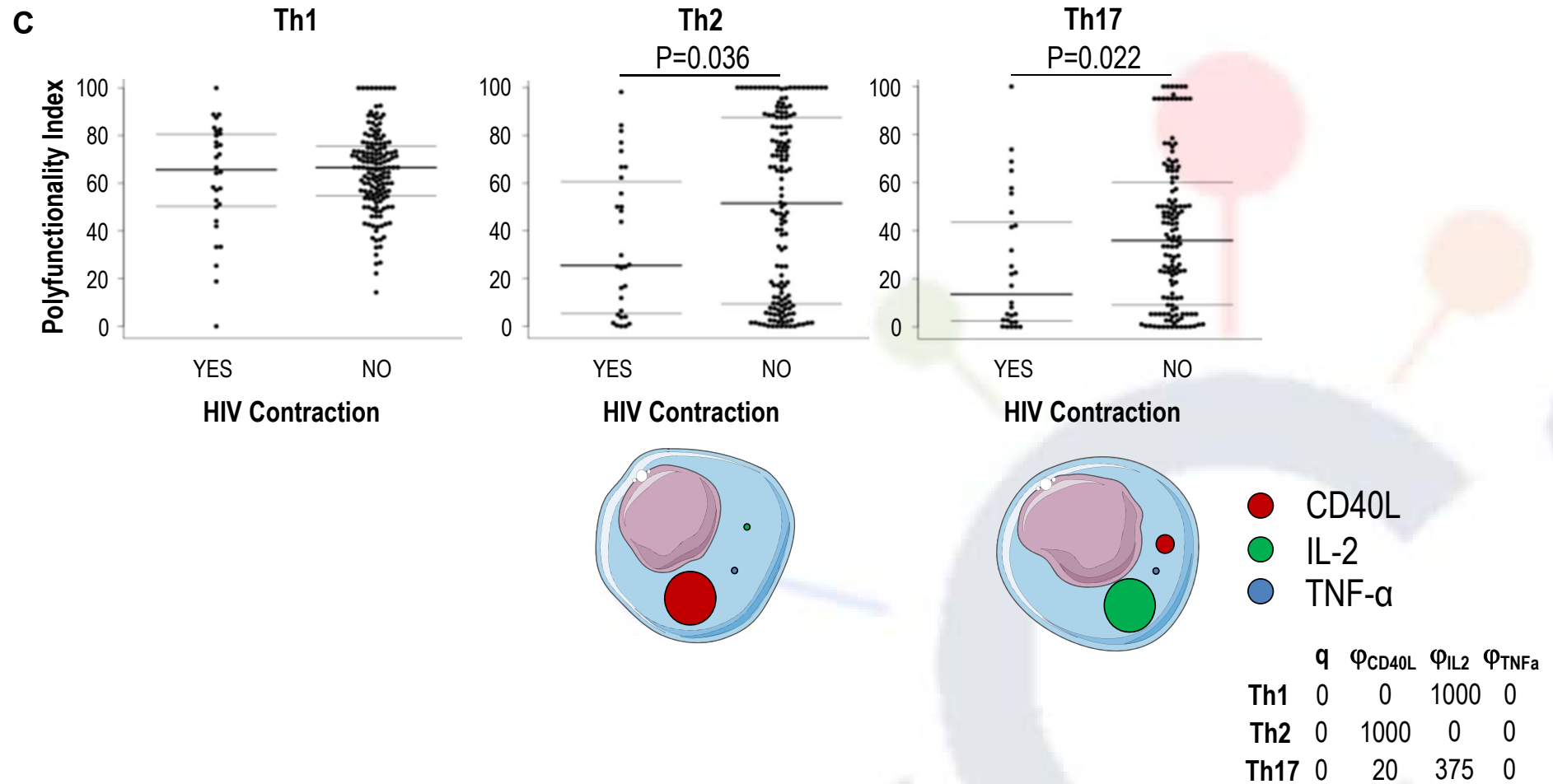
## RV144 HIV case-control clinical trial:



Sauce *et al.* Sci Rep 2016

# CD4<sup>+</sup> T cell polyfunctionality and HIV vaccine protection

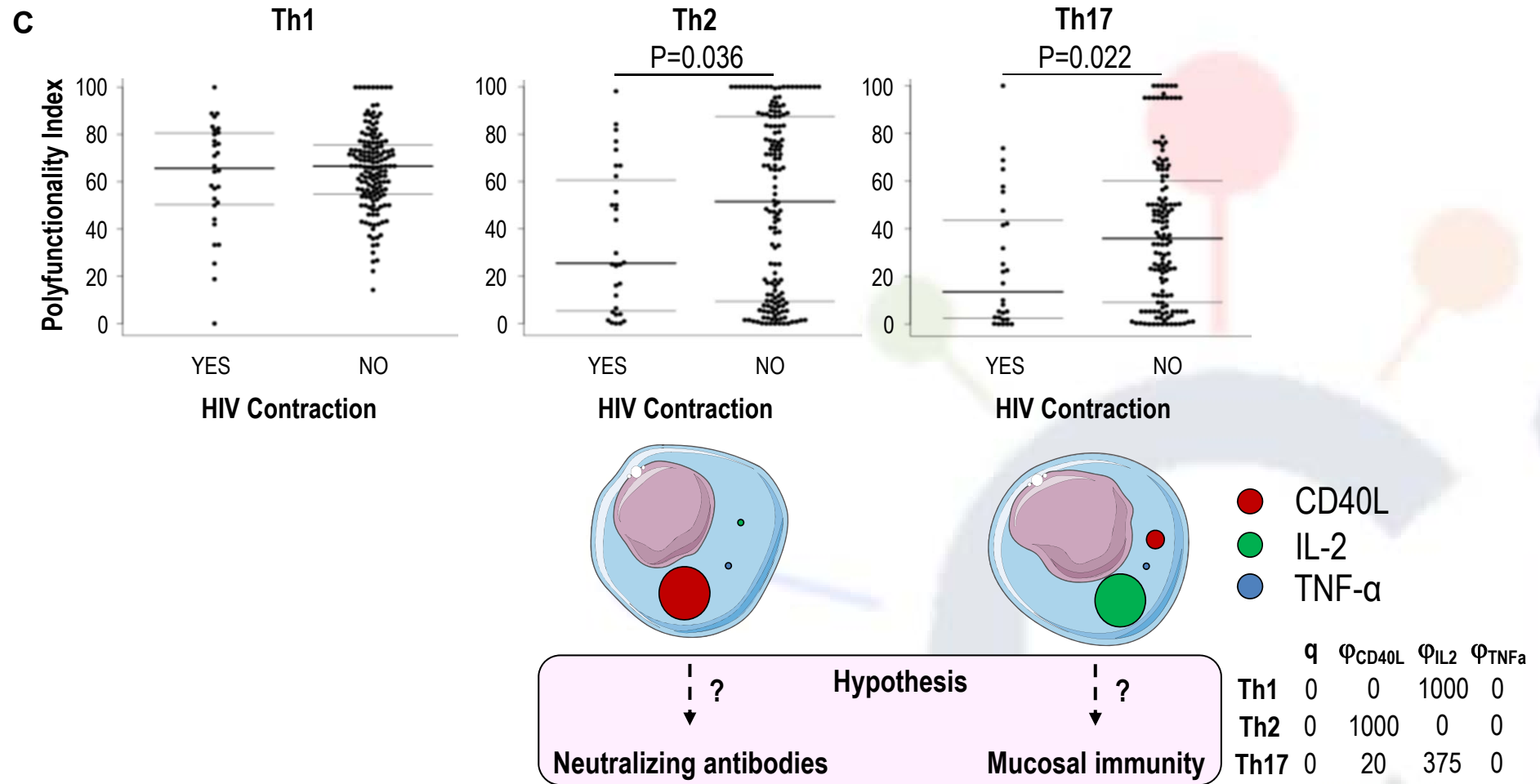
## RV144 HIV case-control clinical trial:



Sauce *et al.* Sci Rep 2016

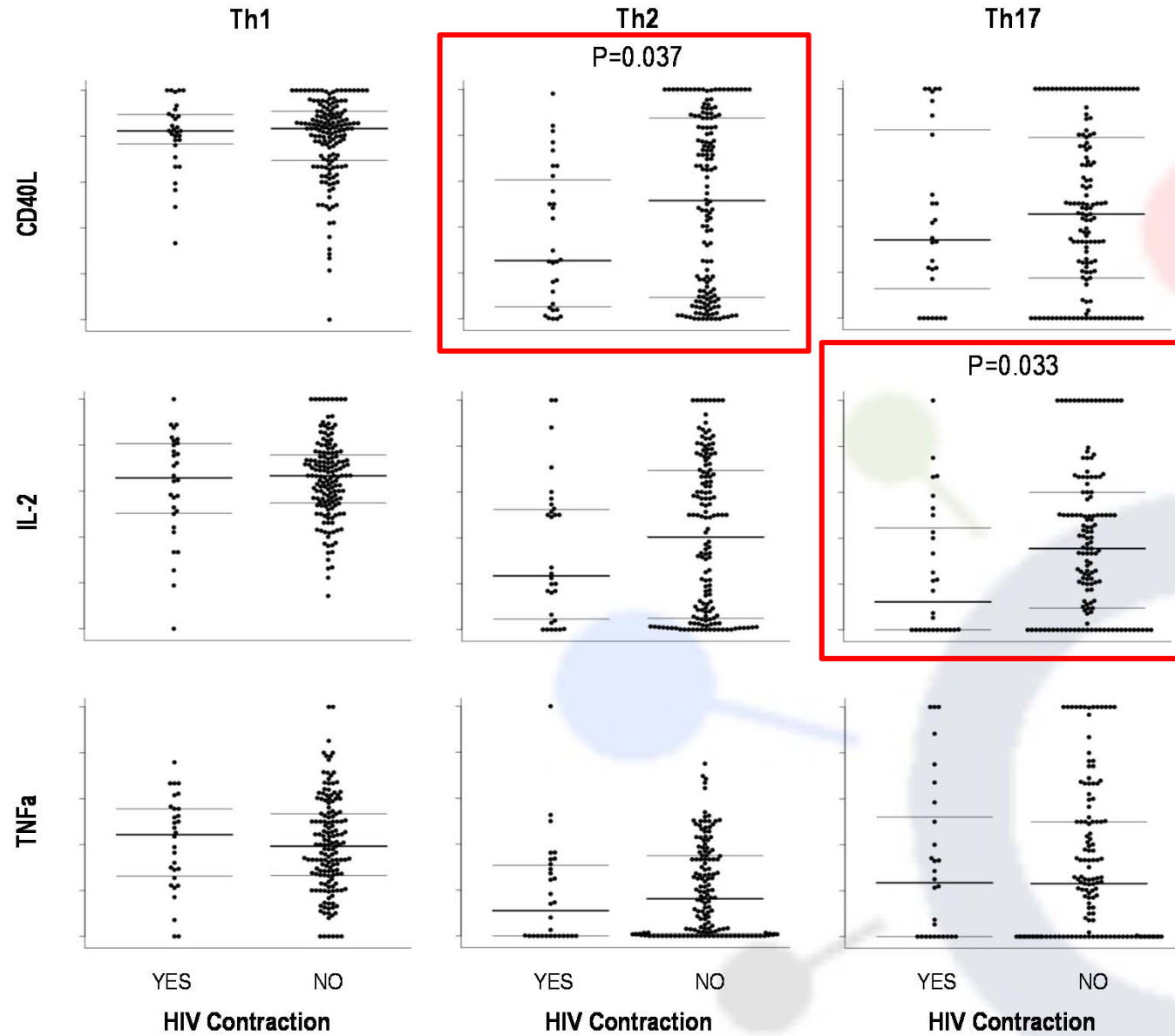
# CD4<sup>+</sup> T cell polyfunctionality and HIV vaccine protection

## RV144 HIV case-control clinical trial:



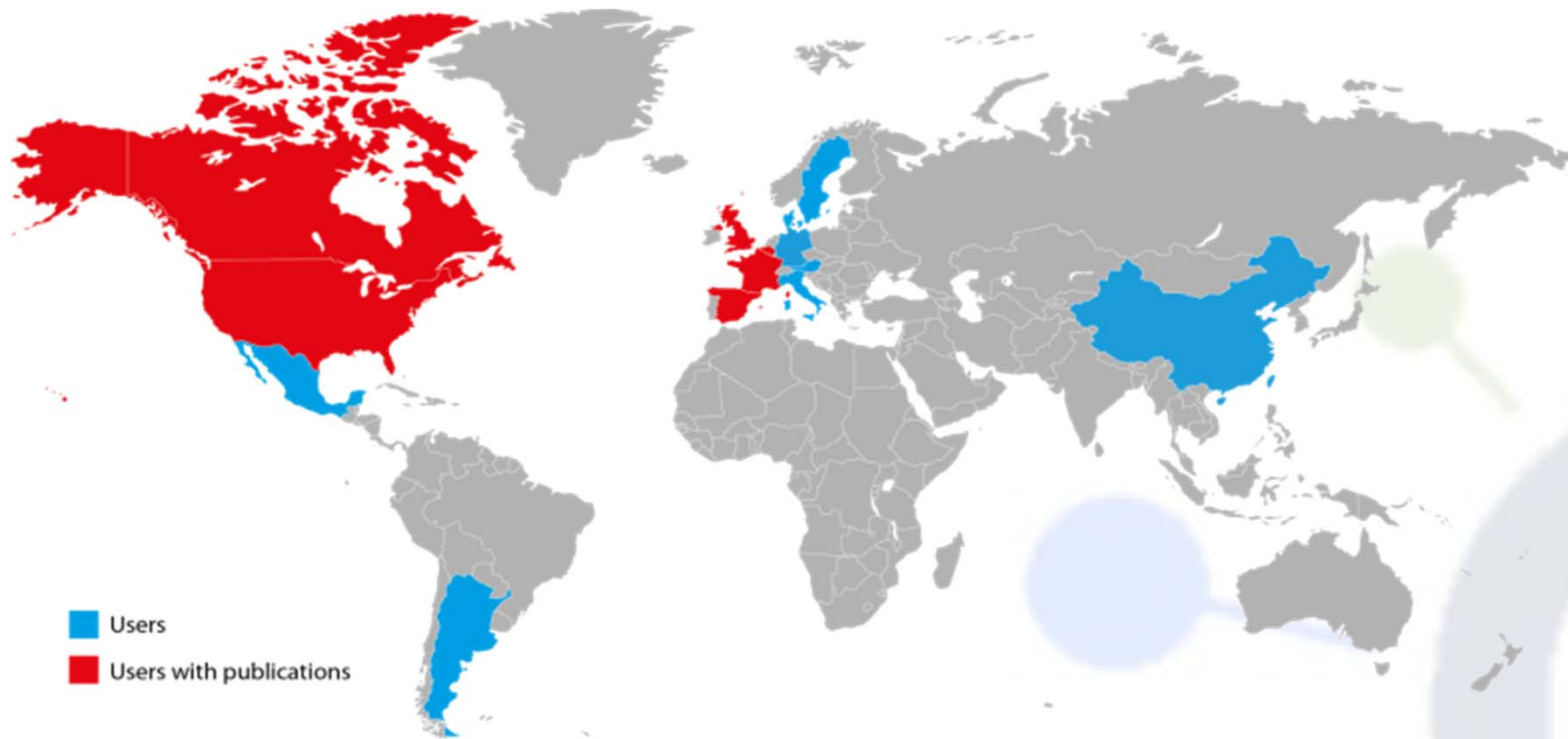
Sauce *et al.* Sci Rep 2016

# CD4<sup>+</sup> T cell polyfunctionality and HIV vaccine protection



Sauce *et al.* Sci Rep 2016

# Funky Cells Tool Box software



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