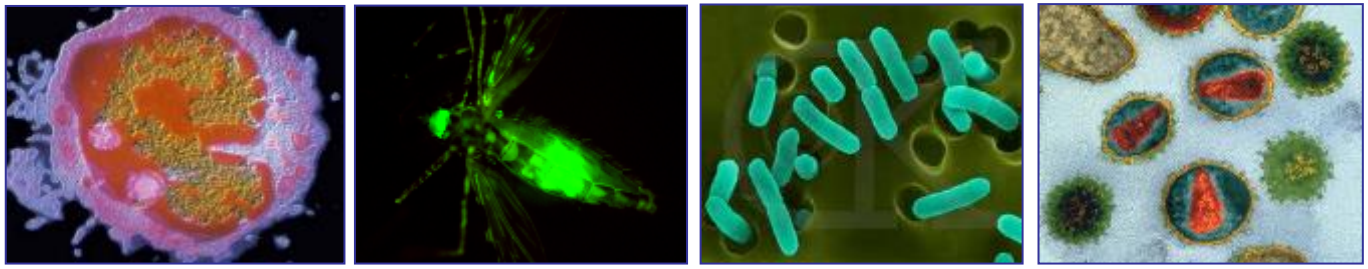


T cell polyfunctionality – an important immune correlate for T cell efficacy and vaccine protection

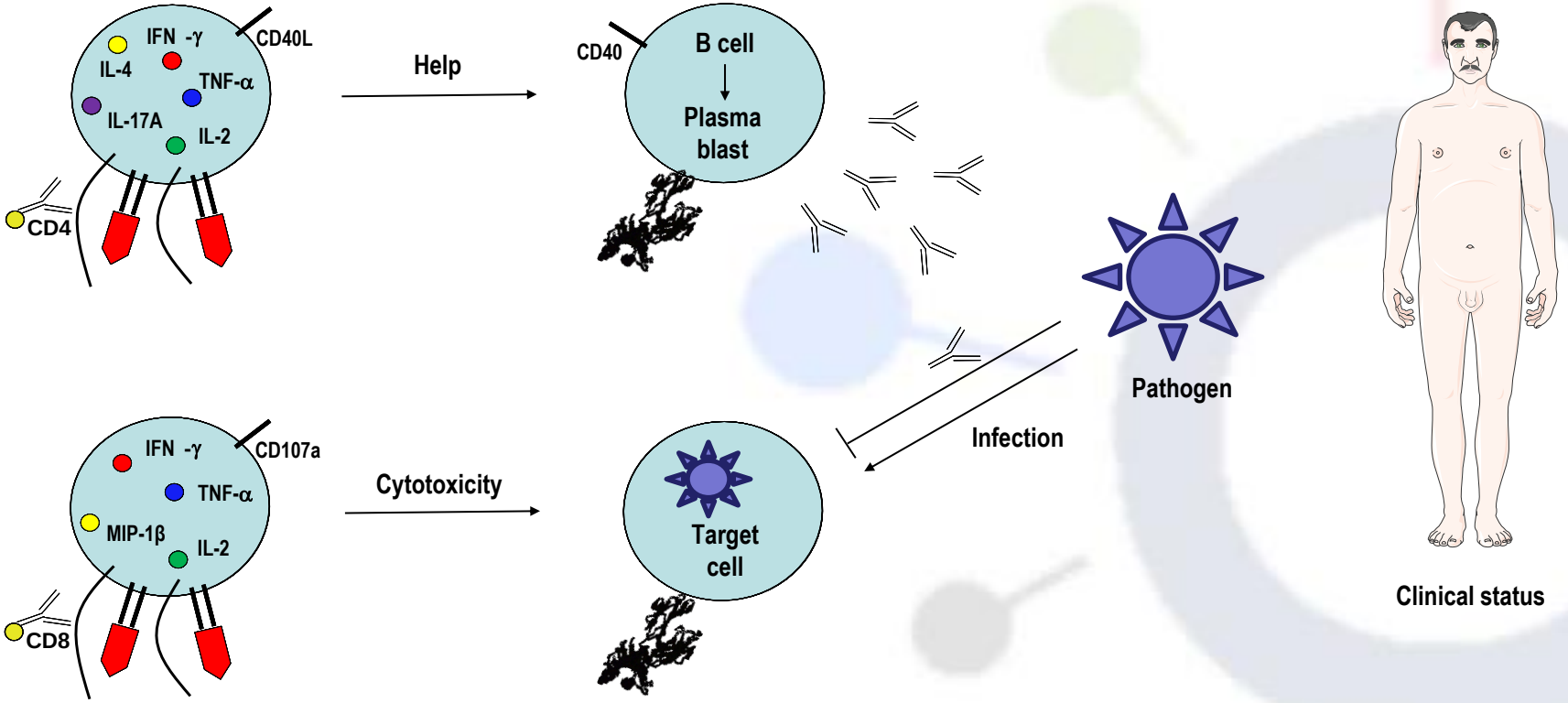
Martin LARSEN

INSERM U1135, CHU Pitié-Salpetrière, Paris, France

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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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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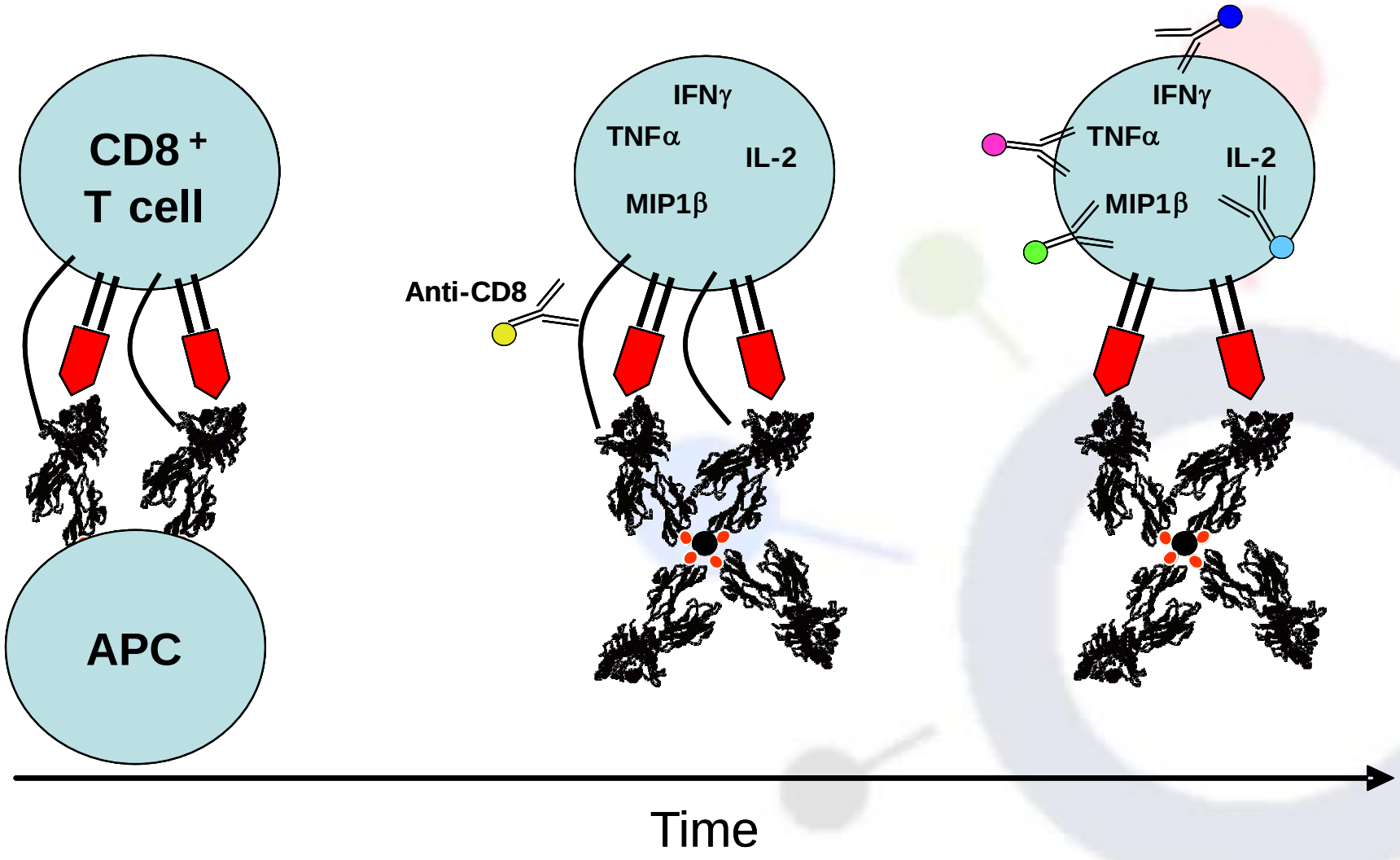
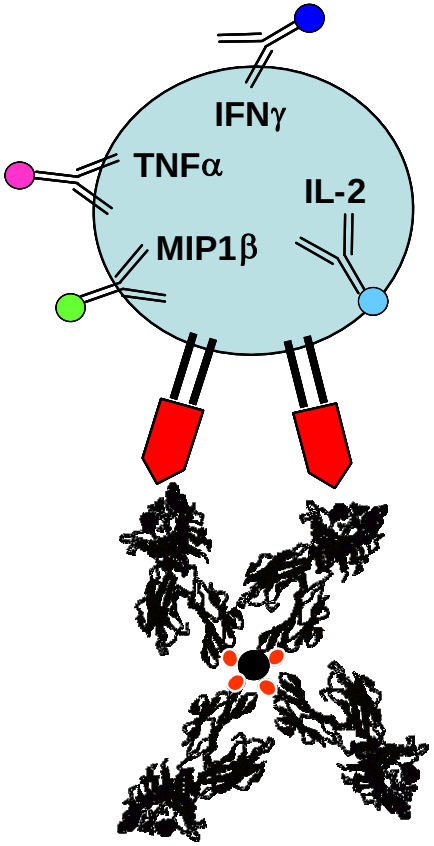
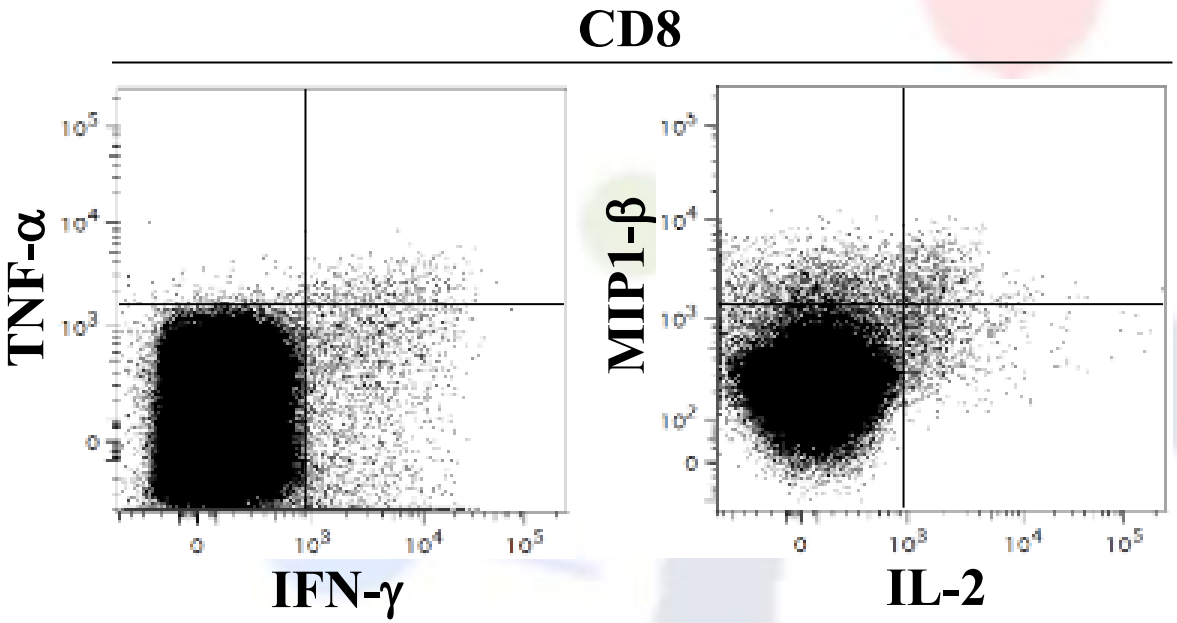
BUT FIRST AN INTRODUCTION TO T CELL POLYFUNCTIONALITY……
How to : Measure, analyse and model

Functional T cell analysis

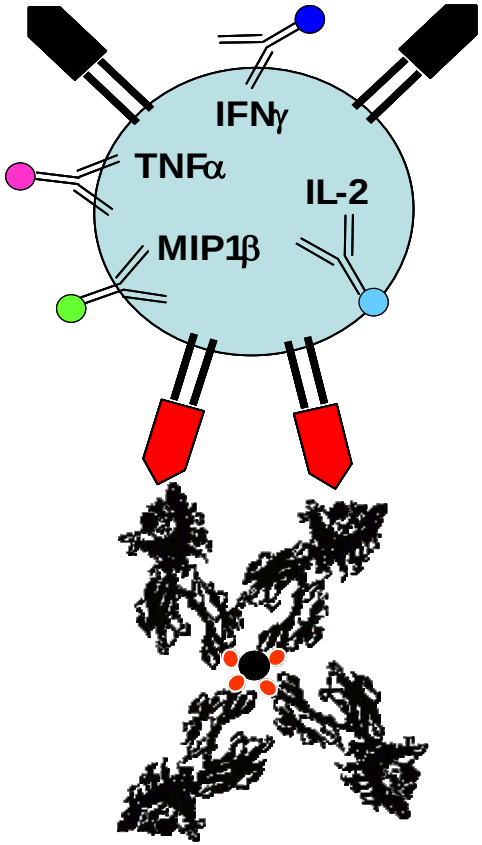


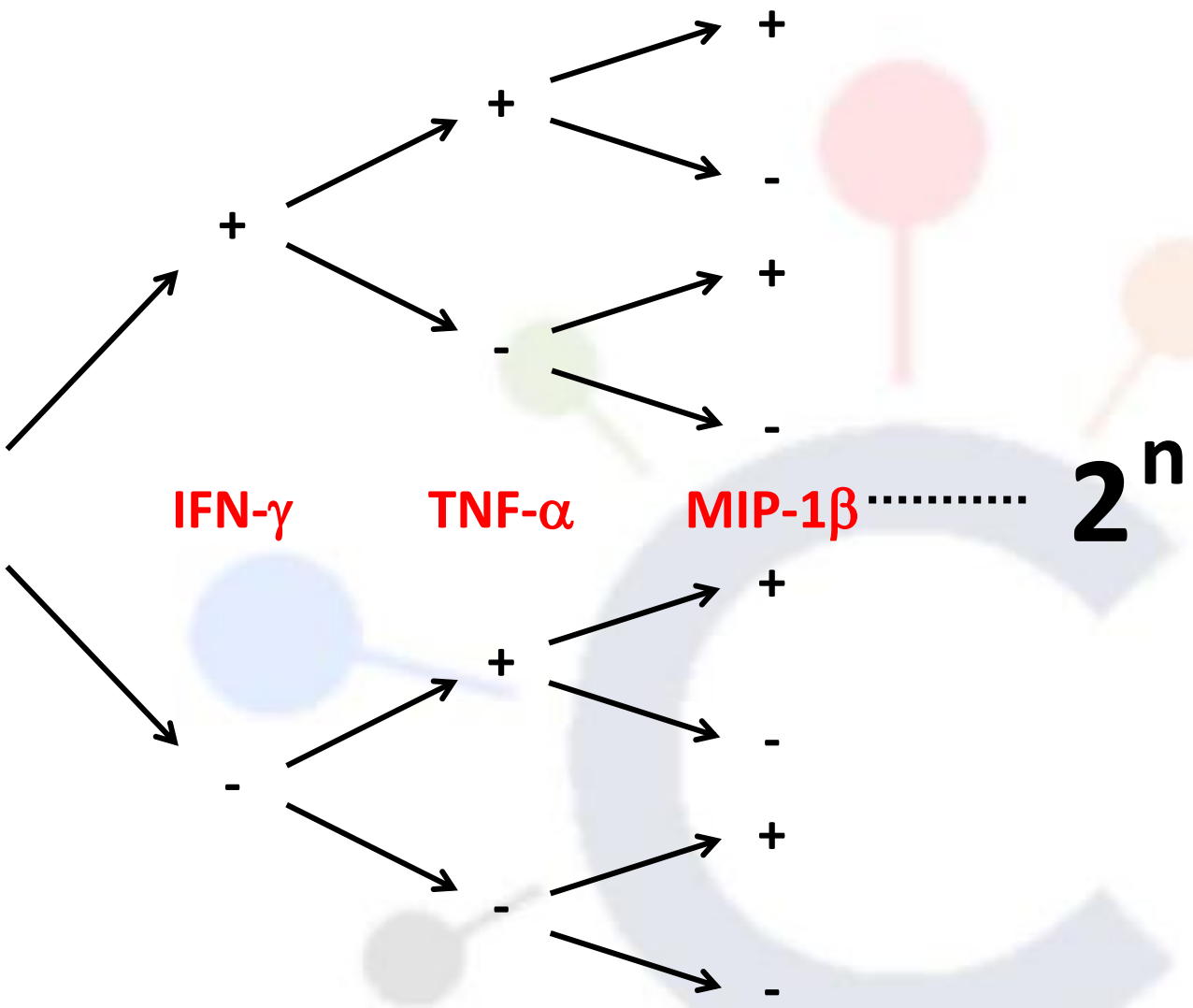
Non-Exhaustive analysis





Exhaustive combinatorial boolean analysis





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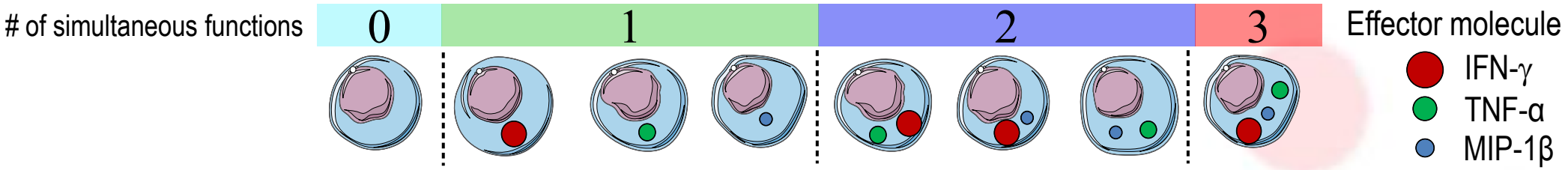
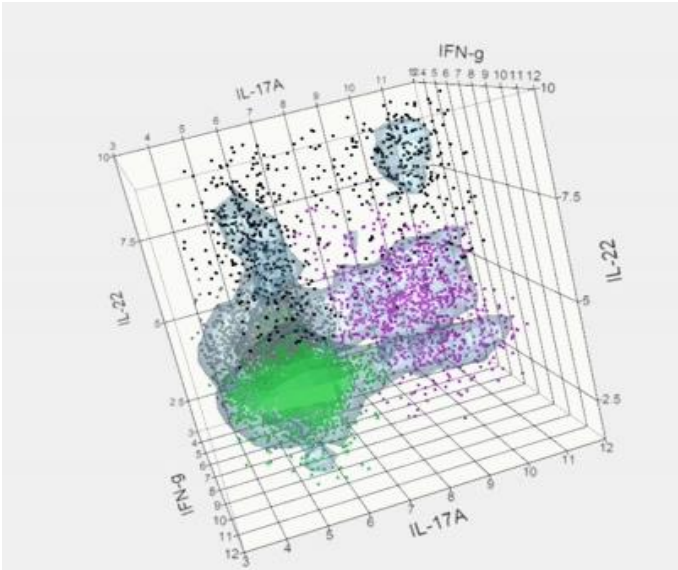
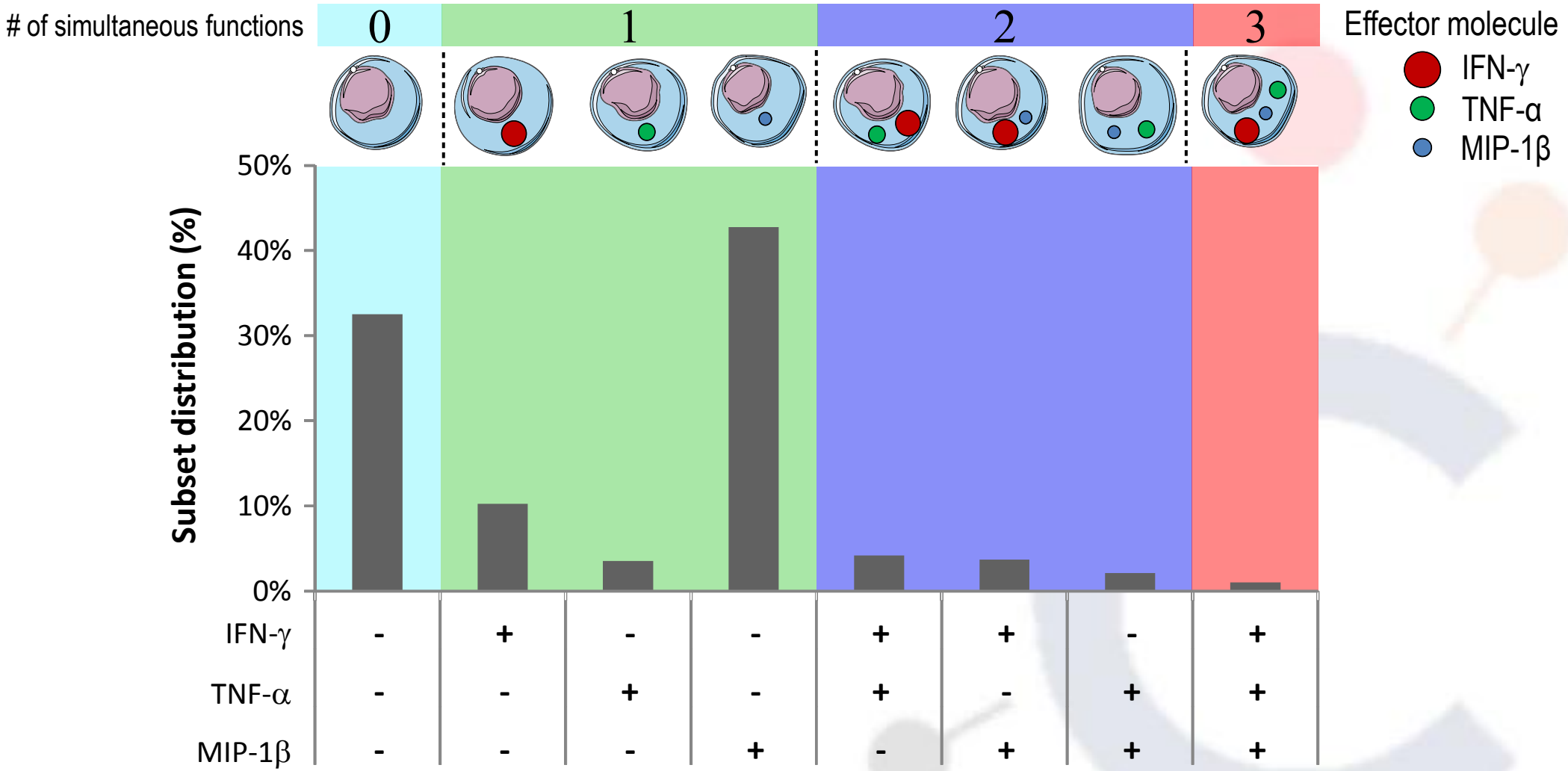




IFN-γ	-	+	-	-	+	+	-	+
TNF-α	-	-	+	-	+	-	+	+
MIP-1β	-	-	-	+	-	+	+	+

Exhaustive combinatorial boolean analysis



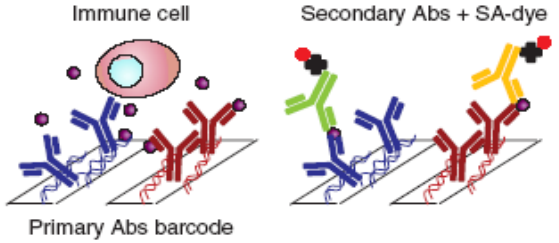
Exhaustive combinatorial boolean analysis

TECHNICAL REPORTS

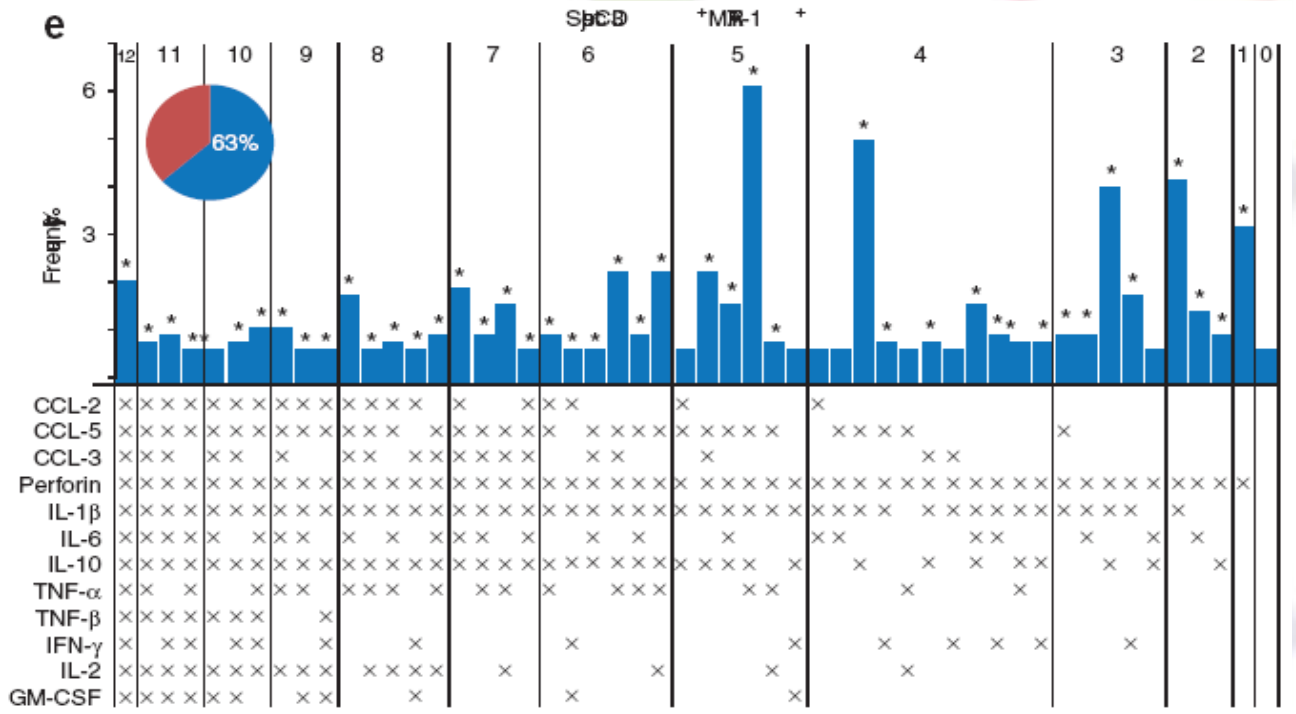
nature medicine

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

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

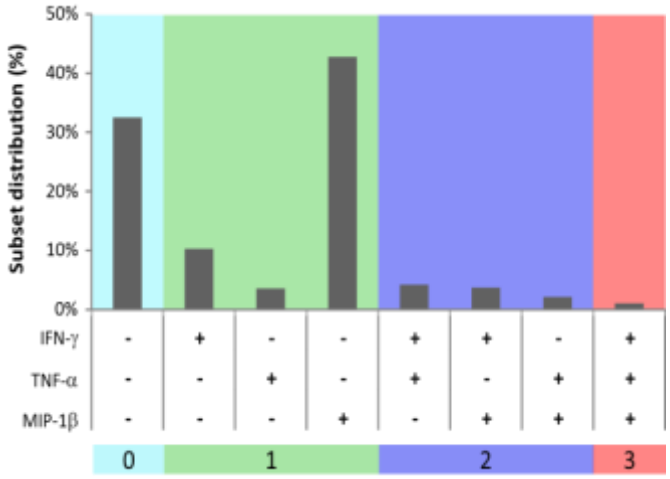


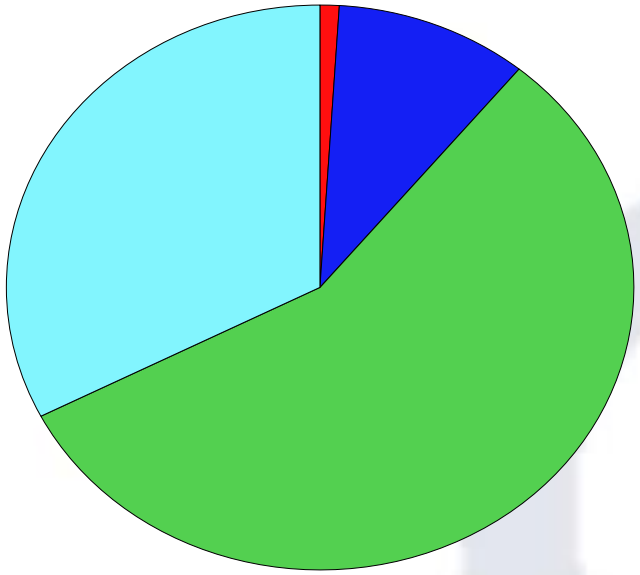
Single-cell secretomics.



Ma *et al.* Nat Med 2011

Qualitative polyfunctionality

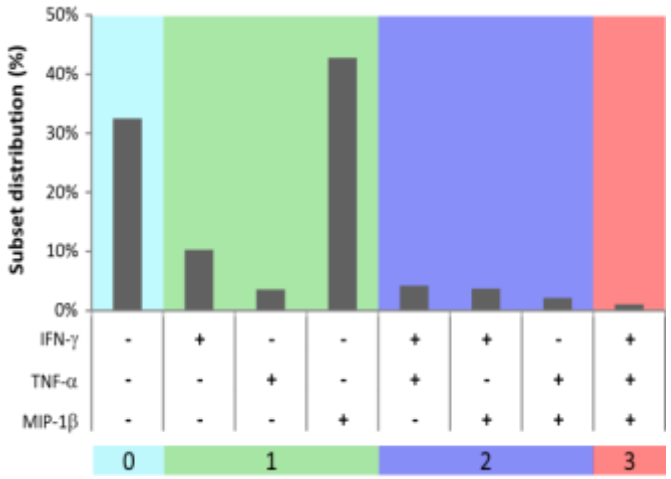




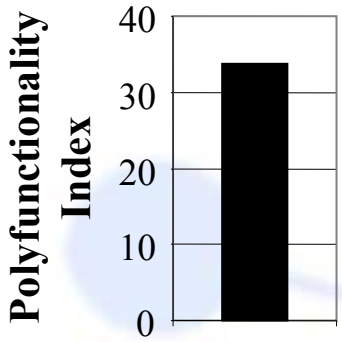
Quantitative polyfunctionality

Polyfunctionality Index

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



Index (1 dimension)



Larsen *et al.* PLoS One 2012

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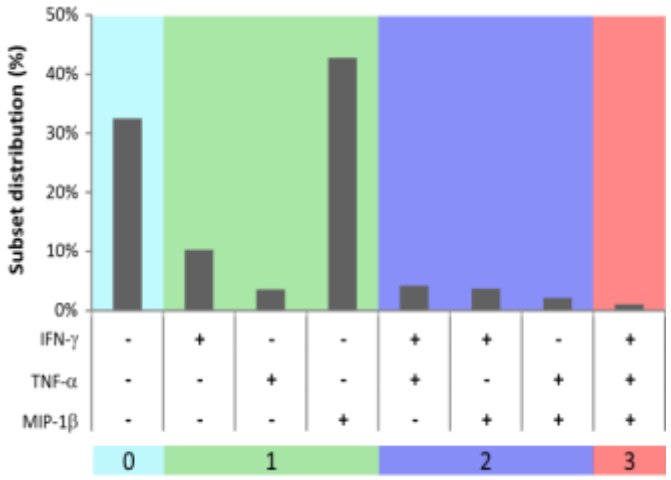
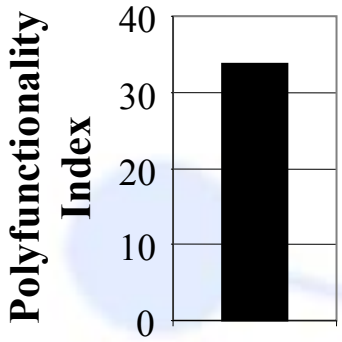
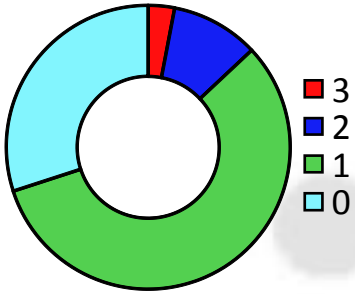
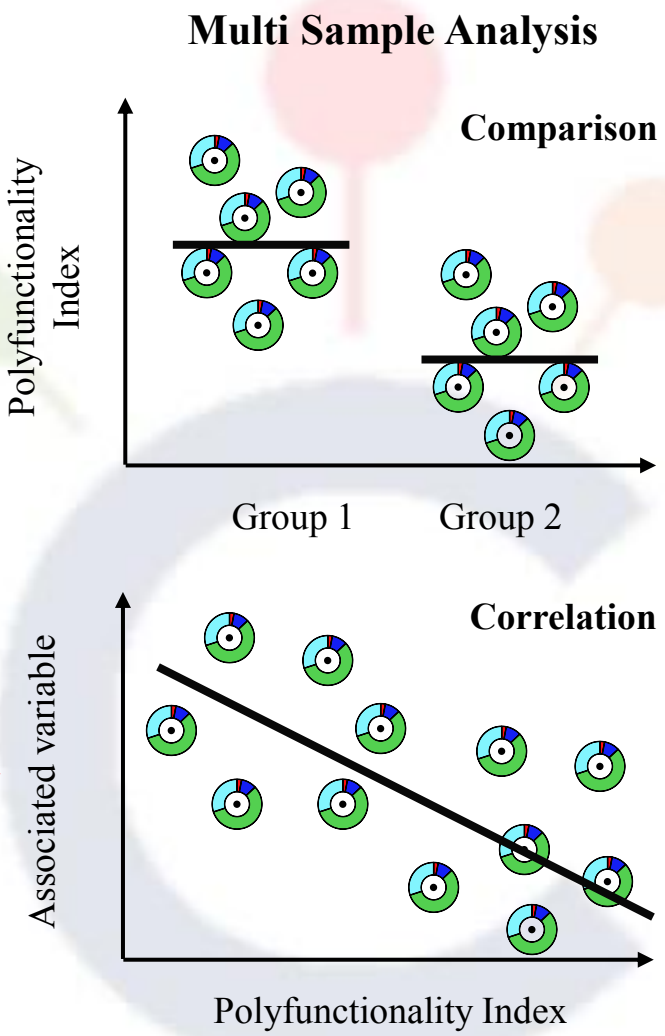


Index (1 dimension)





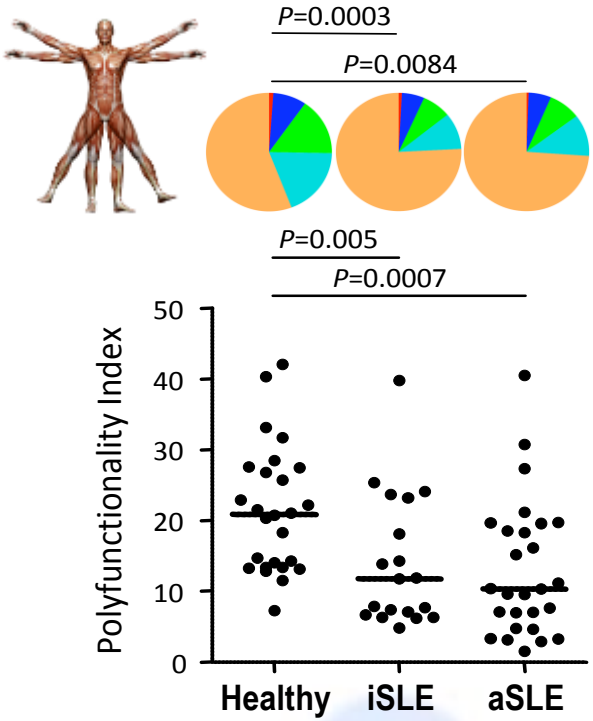
Multi Sample Analysis



Larsen *et al.* PLoS One 2012

Applications of the Polyfunctionality Index

Ex vivo T cell analysis

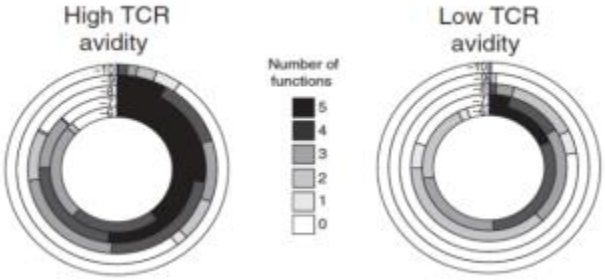


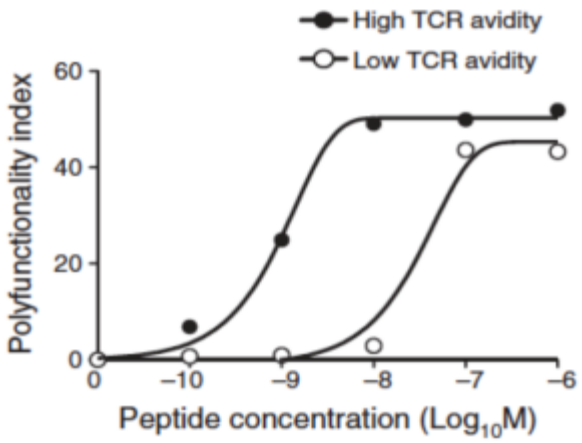
- PD-1^{hi} dysfunctional EBV-specific T cells in SLE patients.

Larsen *et al.* PLoS One 2012
Goulenok *et al.* AIDS 2014
Huygens *et al.* JID 2015
Samri *et al.* AIDS 2016
Bajwa *et al.* JID 2016 and 2017
Cardinaud *et al.* EJI 2017
Antoine *et al.* JID 2014
Lin *et al.* Nat Biotech 2015
Pera *et al.* JLB 2016
Detienne *et al.* Sci Rep 2016
Culina *et al.* Sci Immunol 2018

Applications of the Polyfunctionality Index

In vitro T cell analysis

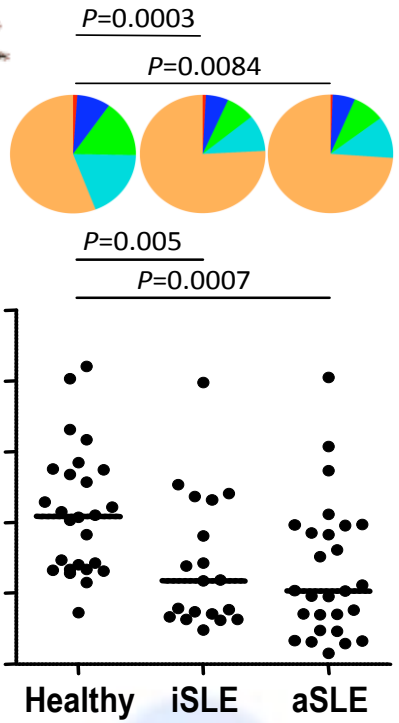




- T cell affinity analysis

Lissina *et al.* AIDS 2014
Hill *et al.* JI 2014
Lissina *et al.* JI 2016
Abdel-Hakeem *et al.* PLoS Path 2017
Burel *et al.* JCI Insight 2017

Ex vivo T cell analysis



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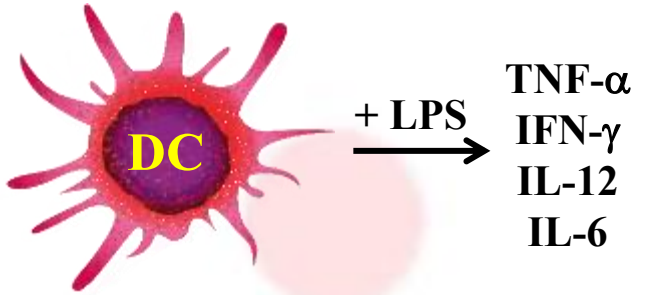
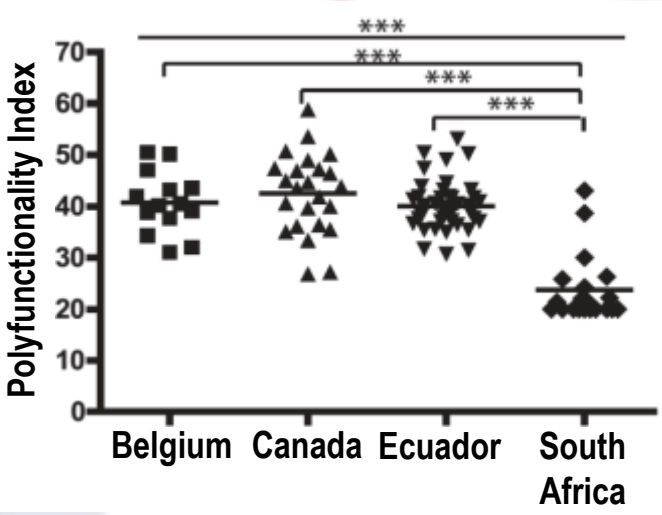
Detienne *et al.* Sci Rep 2016
Culina *et al.* Sci Immunol 2018

Other cell types

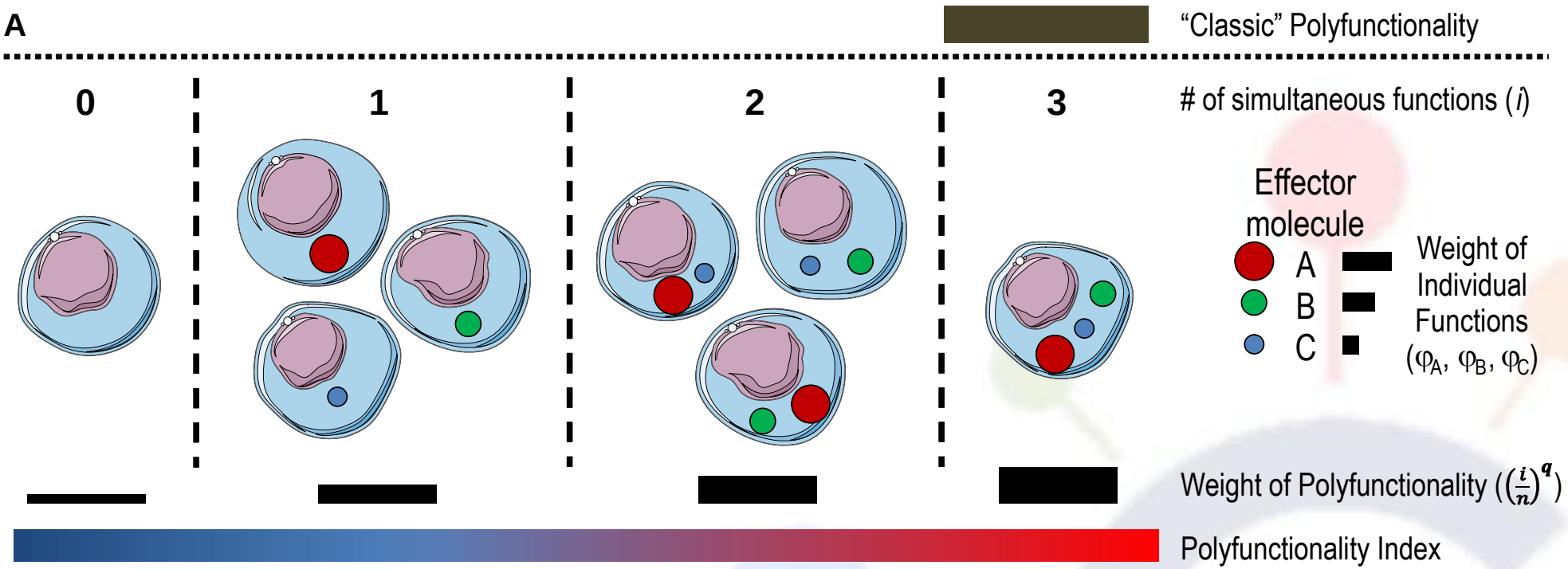




- Dysfunctional DCs from South-African children associated with weak vaccination efficacy.

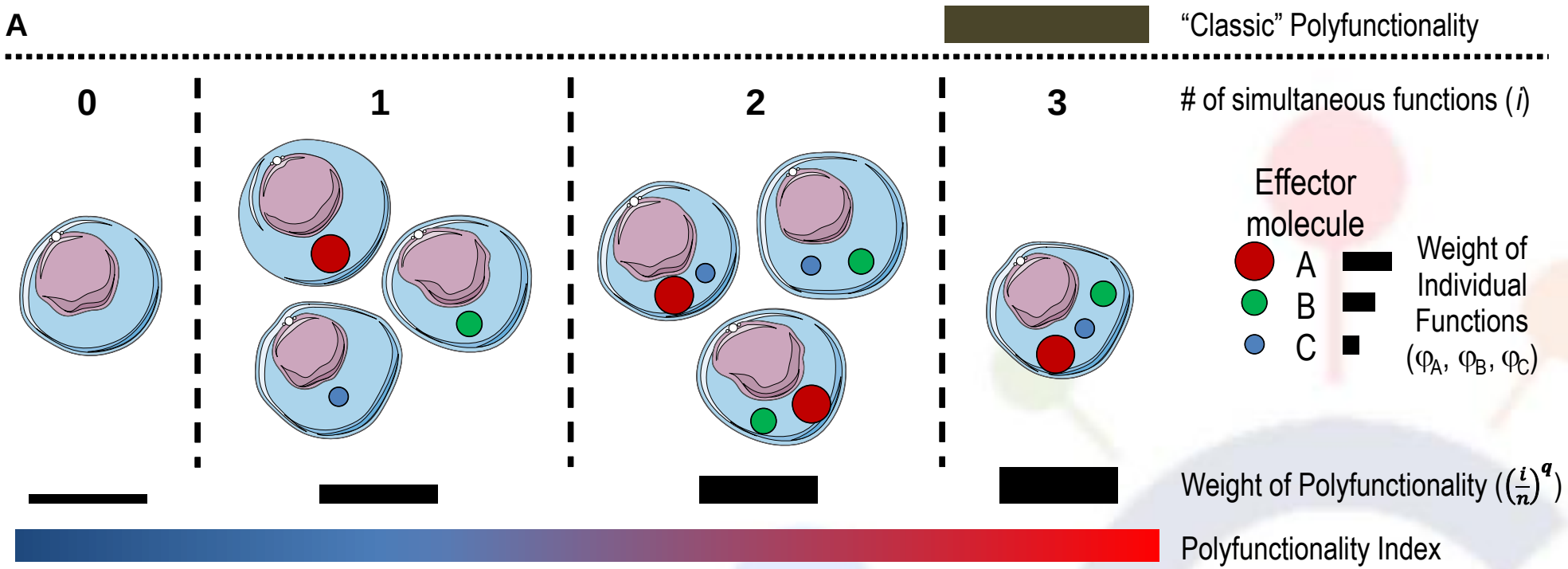
Smolen *et al.* JI 2014
Bayard *et al.* EJI 2016

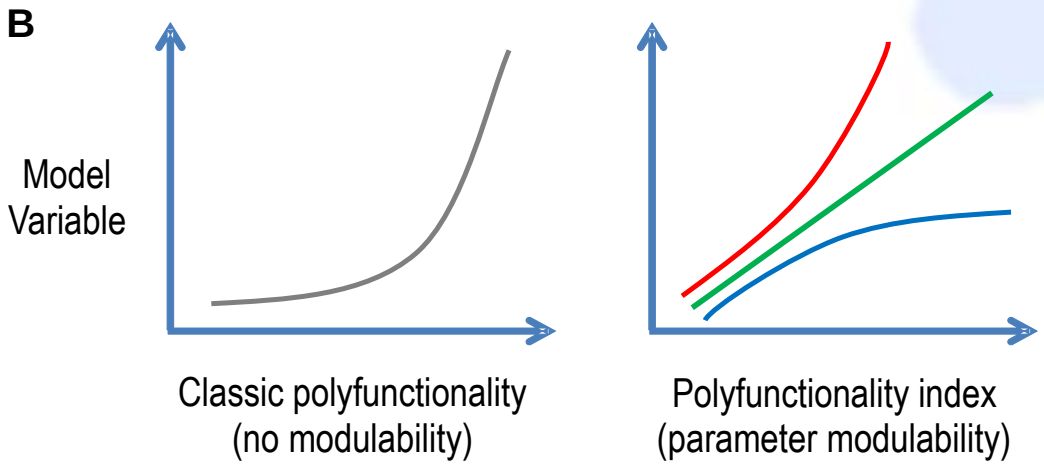
Parametrisation of the Polyfunctionality Index



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

Parametrisation of the Polyfunctionality Index





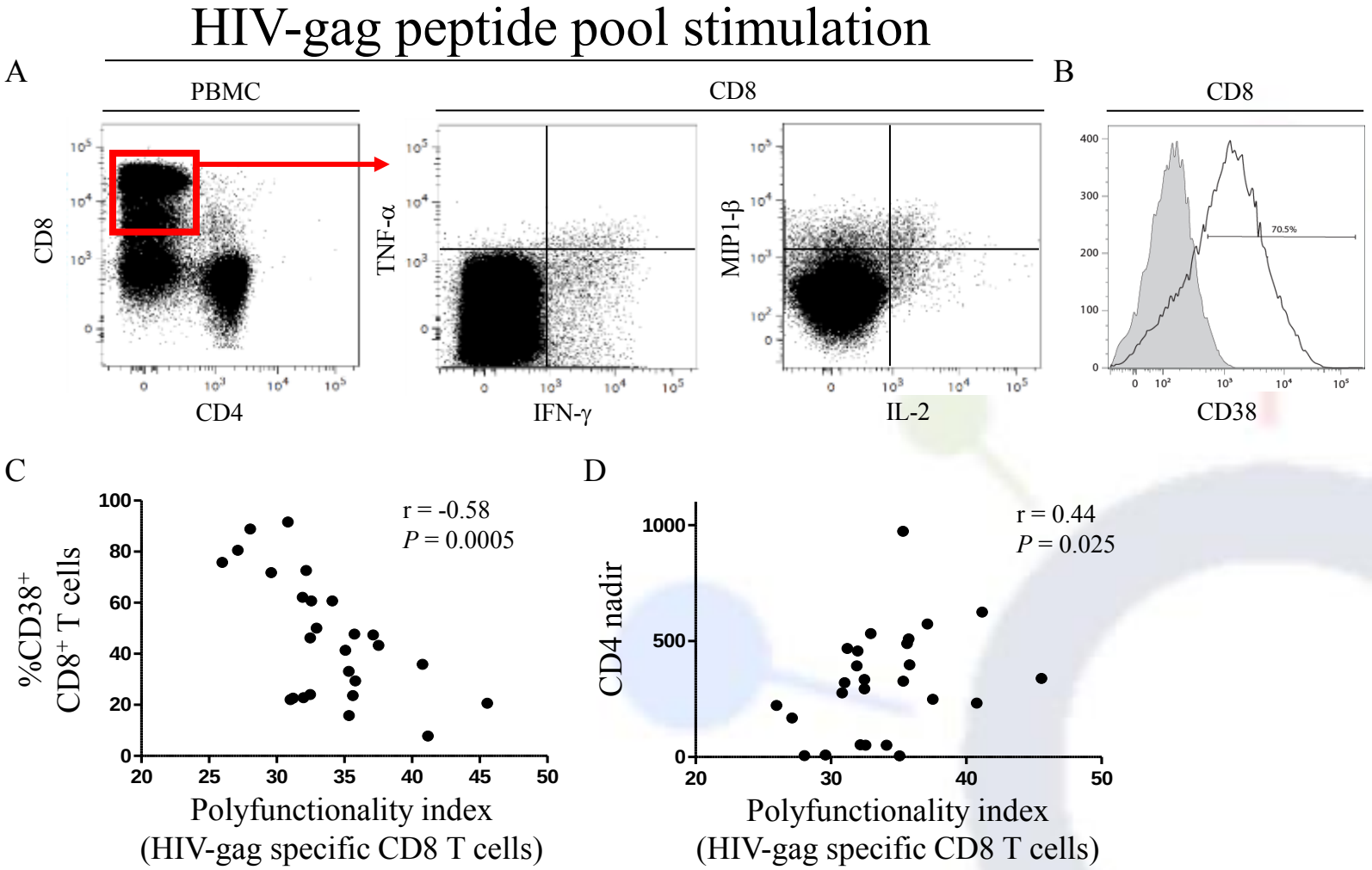
Regression analysis

C

Parameter estimates with inherent biological significance.
φ_A, φ_B, φ_C and q

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

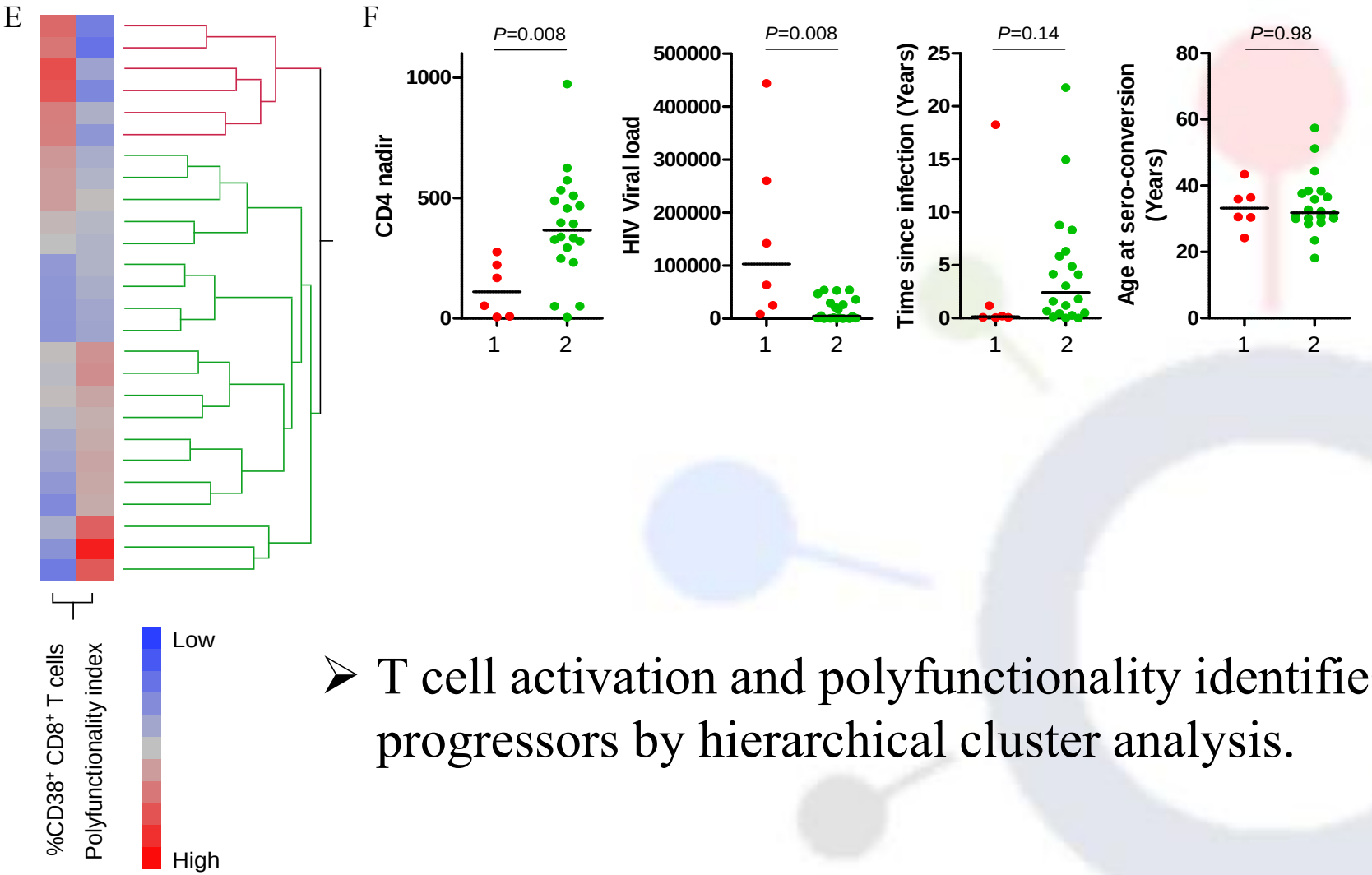
Polyfunctionality of HIV-gag specific CD8+ T cells



- HIV-specific T cell polyfunctionality is negatively correlated with cellular activation (exhaustion) and positively correlated with nadir (lowest $CD4^+$ T cell count).

Larsen *et al.* PLoS One 2012

Polyfunctionality of HIV-gag specific CD8+ T cells



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

Larsen *et al.* PLoS One 2012

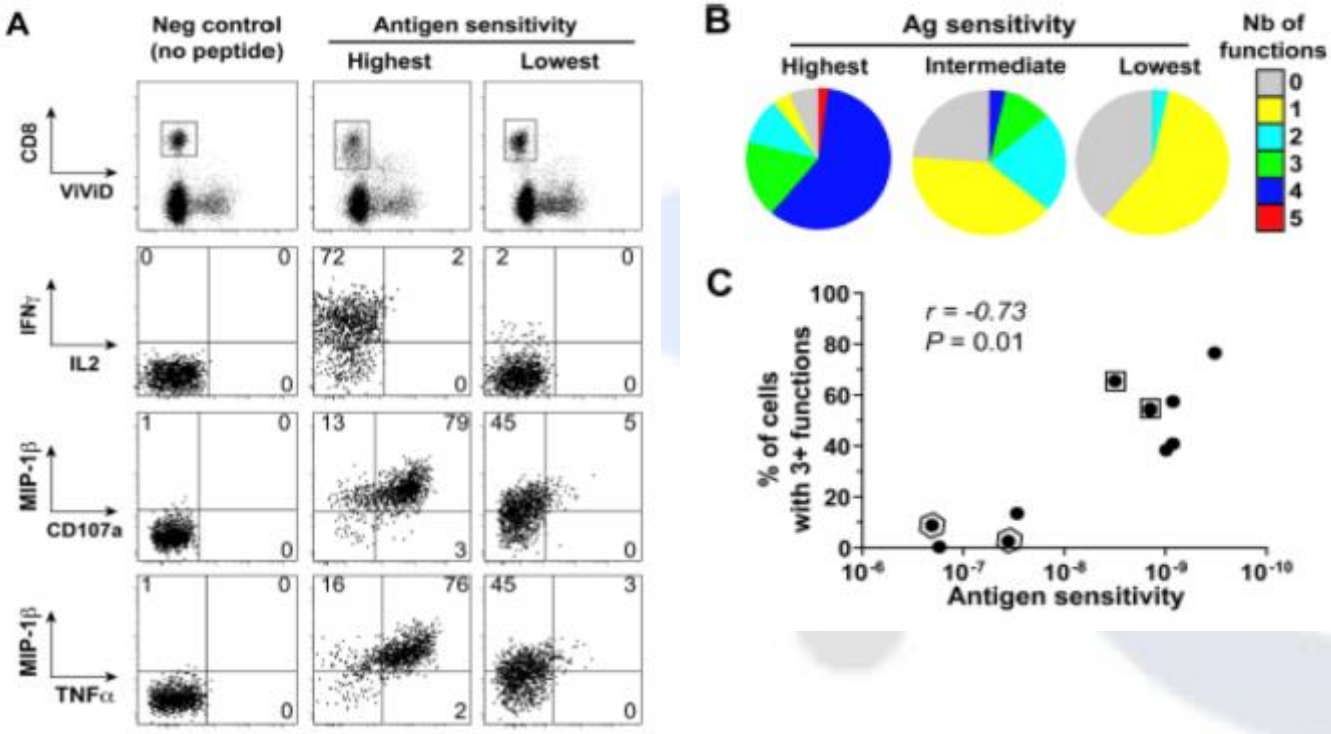
Polyfunctionality of HIV-gag specific CD8+ T cells

blood

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

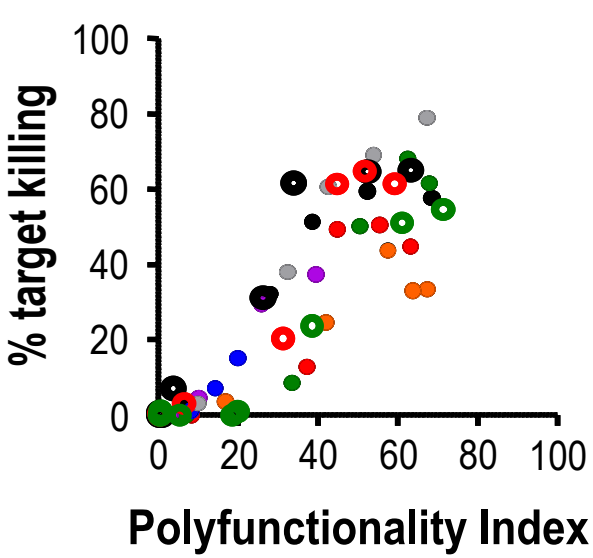
Antigen sensitivity is a major determinant of CD8+ T-cell polyfunctionality and HIV-suppressive activity

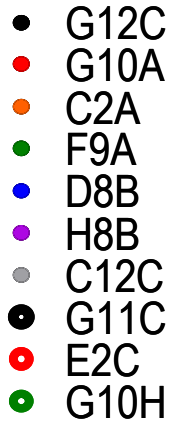
Jorge R. Almeida,[1] Delphine Sauce,[1] David A. Price,[2,3] Laura Papagno,[1] So Youn Shin,[4] Arnaud Moris,[5] Martin Larsen,[1] Gianfranco Pancino,[4] Daniel C. Douek,[2] Brigitte Autran,[1] Asier Sáez-Cirión,[4] and Victor Appay[1]

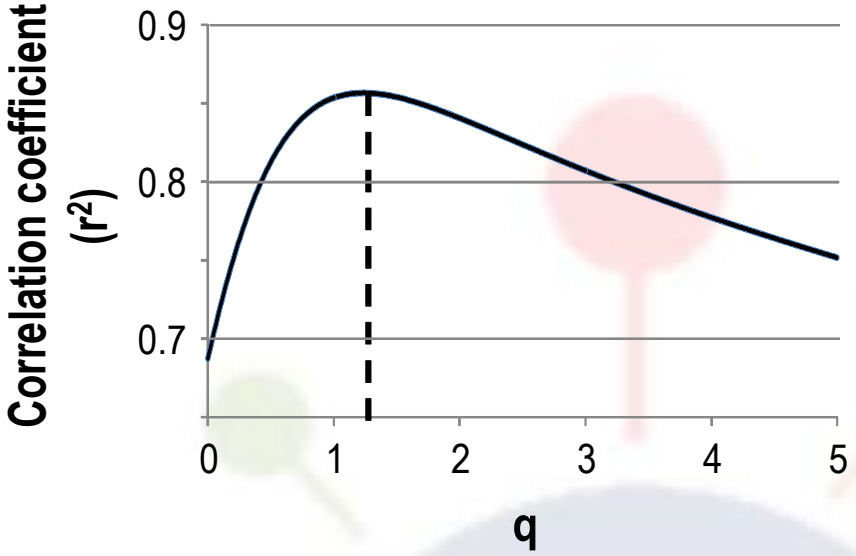


Almeida *et al.* Blood 2009

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.

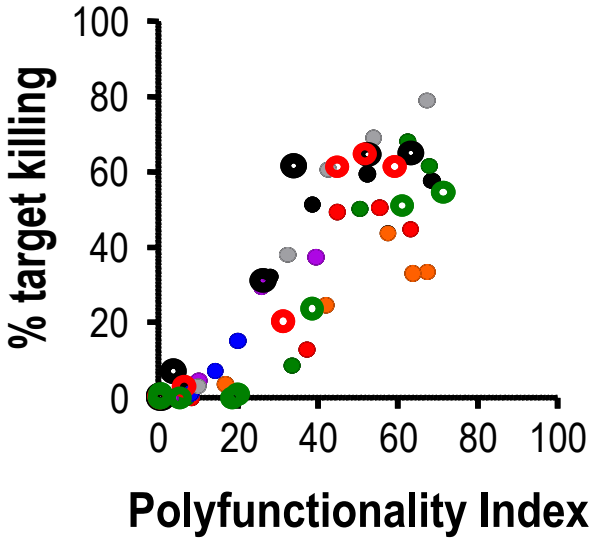
0 < q < 1 : polyfunctionality is a moderate immune correlate.

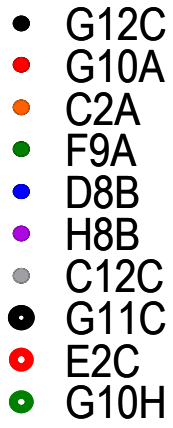
q > 1 : polyfunctionality is a strong immune correlate.

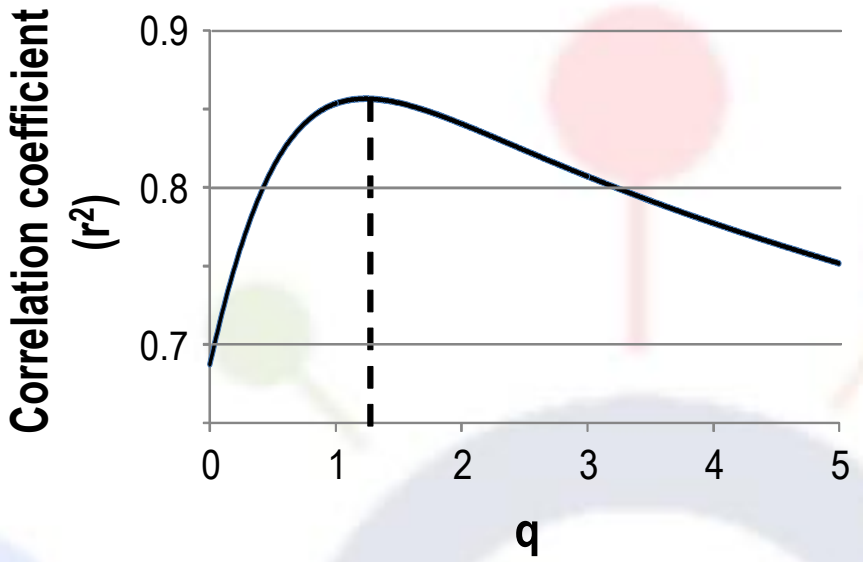
$$PI = \sum_{i=0}^{n} F_i \cdot \left(\frac{i}{n}\right)^q$$

Boyd *et al.* PLoS One 2015

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







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

Parameters	Model 1 Estimate (95% CI)	*p*	Model 2 Estimate (95% CI)	*p*
q	1.24 (0.85, 1.63)	**<0.001**	3.05 (1.30, 4.81)	**0.001**
ϕ				
CD107a	--		-0.96 (-3.96, 2.03)	0.5
TNF-α	--		-5.65 (-12.38, 1.08)	0.10
IFN-γ	--		1.04 (-0.36, 2.44)	0.14
MIP-1β	--		5.86 (-4.32, 16.03)	0.3
IL-2	--		-0.62 (-1.62, 0.39)	0.2
Correlation (R^2)	0.8563		0.9144	

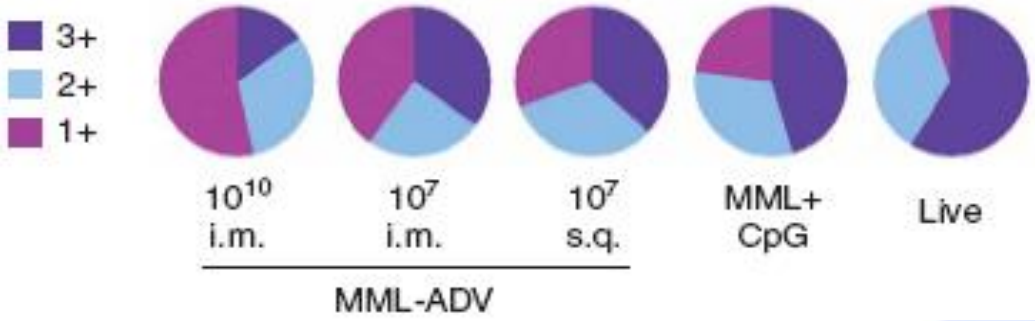
Boyd *et al.* PLoS One 2015

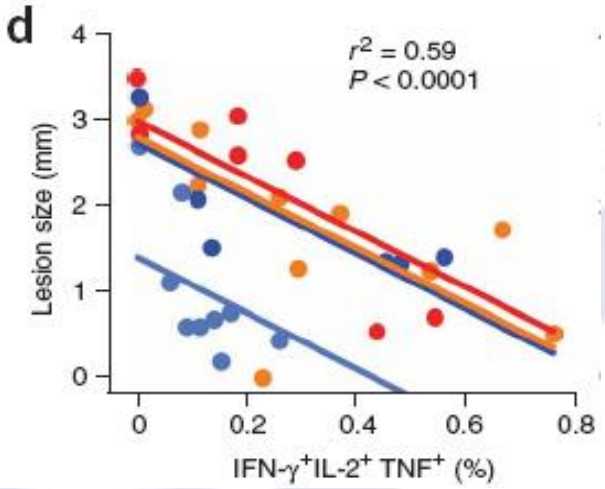
Polyfunctionality is a correlate of Leishmania major vaccine efficacy *in vivo*

nature medicine

Multifunctional T_H1 cells define a correlate of vaccine-mediated protection against *Leishmania major*

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



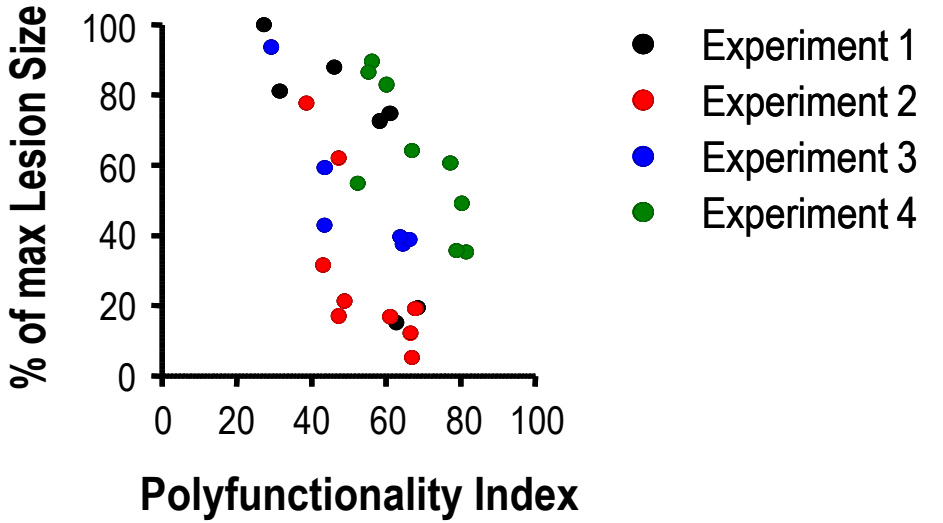


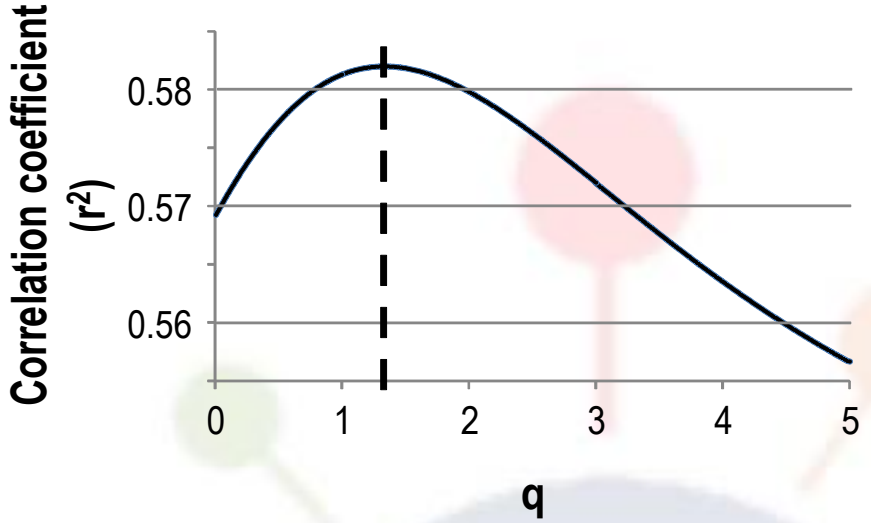
Corresponds to special PI with q = ∞

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:

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.

Boyd *et al.* PLoS One 2015

RV144 trial design and output

RV144 HIV case-control clinical trial:

Inclusion of 16.402 HIV sero-negative individuals from a high-risk population

W0 — ALVAC HIV (vCP1521)

W4 — ALVAC HIV (vCP1521)

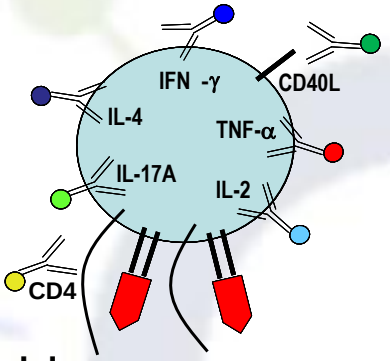
W12 — ALVAC HIV (vCP1521) + AIDSVAX B/E

W24 — ALVAC HIV (vCP1521) + AIDSVAX B/E

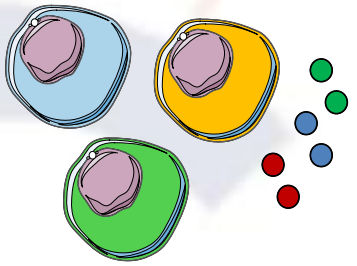
W26 — Blood samples - Polyfunctionality analysis of 226 vaccinated individuals.

M42 — **Clinical follow-up - HIV Contraction**
Among 16.402 inclusions, 51 vaccinees and 74 placebo individuals contracted HIV. 41 vaccinated HIV contractors were included in the present study.

Intracellular CD4 T cell Polyfunctionality



Other biomarkers (cellular and soluble)



Prediction models
CD4 polyfunctionality

Li *et al.* Nat Biotech 2015,

Prediction models
Anti-ENV IgG (positive)
Anti-ENV IgA (negative)

Rerks-Ngarm *et al.* NEJM 2009,
Haynes *et al.* NEJM 2012

RV144 trial design and output

RV144 HIV case-control clinical trial:

Inclusion of 16.402 HIV sero-negative individuals from a high-risk population

W0 — ALVAC HIV (vCP1521)

W4 — ALVAC HIV (vCP1521)

W12 — ALVAC HIV (vCP1521) + AIDSVAX B/E

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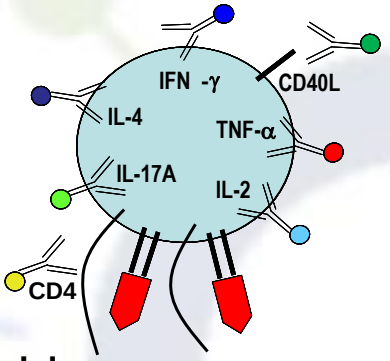

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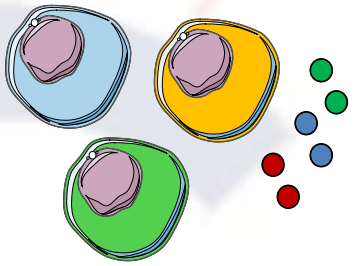

Prediction models
CD4 polyfunctionality
CD40L⁺ Th2 (positive)
IL-2⁺ Th17 (positive)

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

Prediction models
Anti-ENV IgG (positive)
Anti-ENV IgA (negative)

Rerks-Ngarm *et al.* NEJM 2009,
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COMPASS – a Bayesian hierarchical framework model

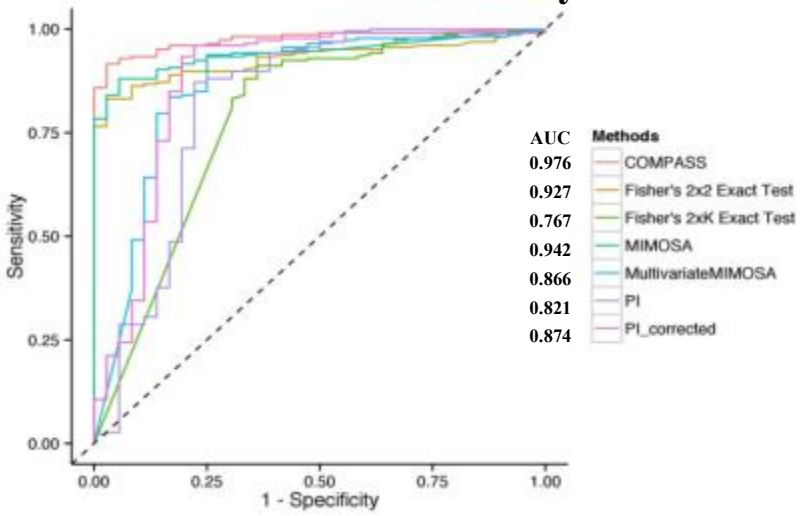
RV144 HIV case-control clinical trial:

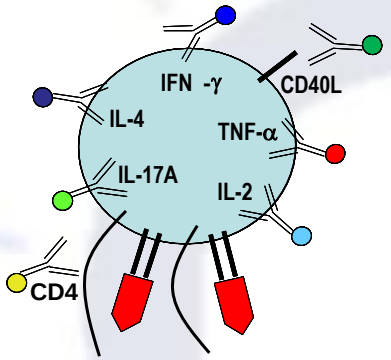
Vaccine: ALVAC HIV (vCP1521) + AIDSVAX B/E (gp120)

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.

RV144 full study





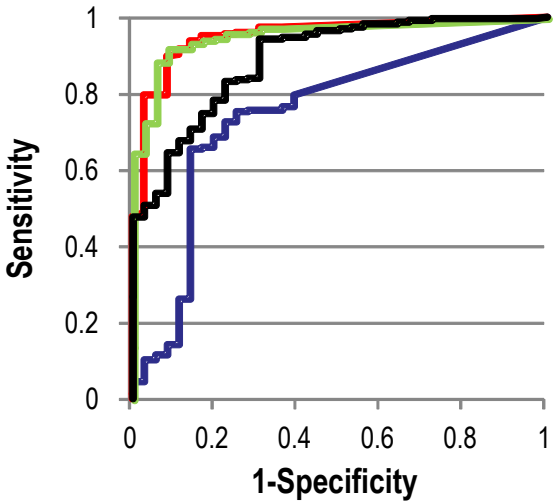
Prediction: Vaccinee vs placebo
Inclusion: 226 vaccinees and 40 placebo
HIV contraction: 41 vaccinees and 20 placebo

Lin *et al.* Nature Biotechnology 2015

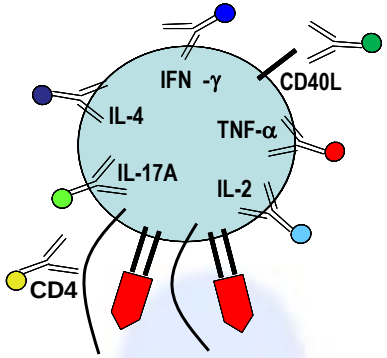
COMPASS – a Bayesian hierarchical framework model

RV144 HIV case-control clinical trial:

A



	AUC
PI (q=37.22)	0.902±0.041
PFS	0.957±0.023
FS	0.951±0.029
Res Size	0.747±0.077



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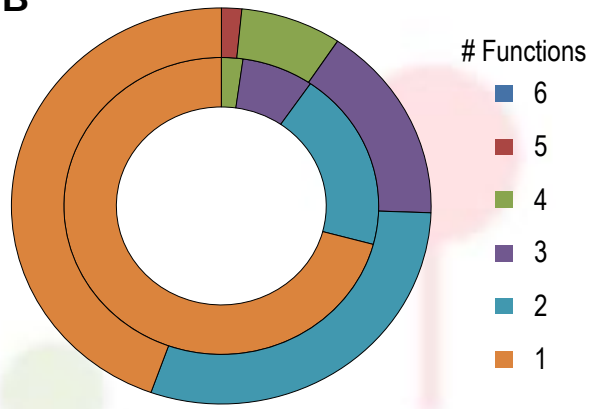

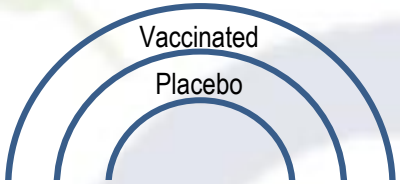

T cell quantity = Response size (applicable to samples with and without HIV-specific T cells).
T cell quality = Polyfunctionality (applicable only to samples with HIV-specific T cells).
Why is response size a less good predictor compared to polyfunctionality?

Sauce *et al.* Sci Rep 2016

COMPASS – a Bayesian hierarchical framework model

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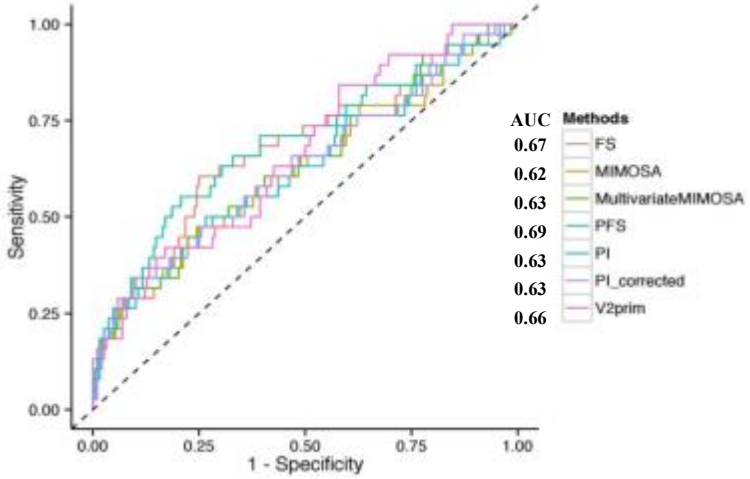

Prediction: HIV contraction
Inclusion: 226 vaccinees and 40 placebo
HIV non-contraction: 185 vaccinees and 20 placebo
HIV contraction: 41 vaccinees and 20 placebo

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-α^+ IFN-γ^+ 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
TNFa+IFNg+IL4+IL2+CD40L+ (P)	0.946(0.66 – 1.35)	0.761	0.959

Lin *et al.* Nature Biotechnology 2015

COMPASS – a Bayesian hierarchical framework model

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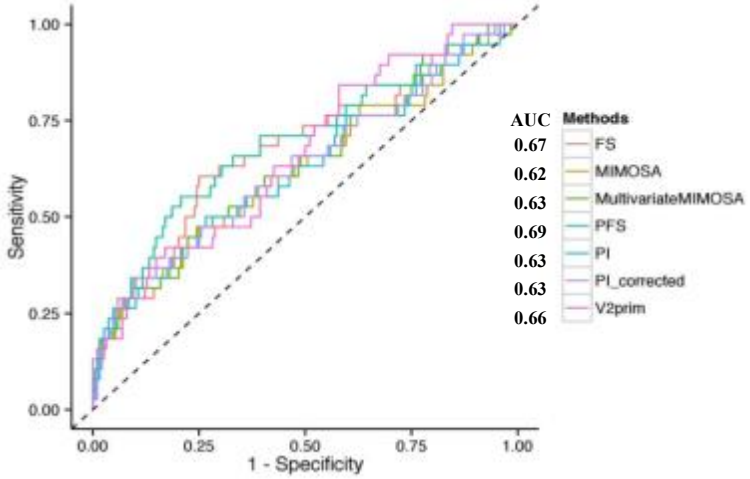

Prediction: HIV contraction
Inclusion: 226 vaccinees and 40 placebo
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1/3 of non-controllers are placebo versus 1/10 in controllers. HUGE BIAS….

Bayesian probability (poly)functionality scores:

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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 Blotechnology 2015

CD4+ 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

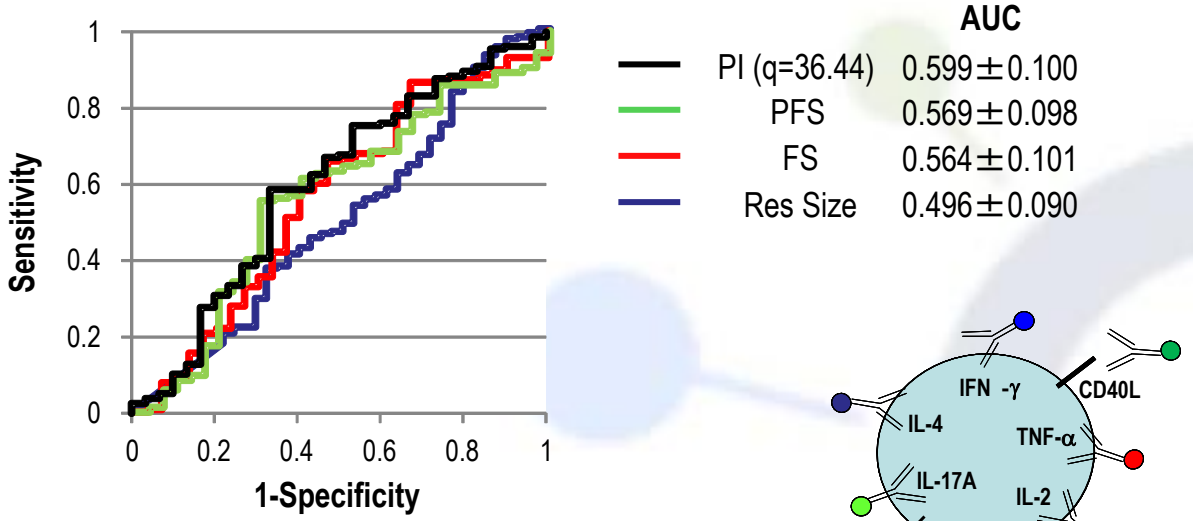
Inclusion: 185 vaccinees with detectable HIV-specific T cells.

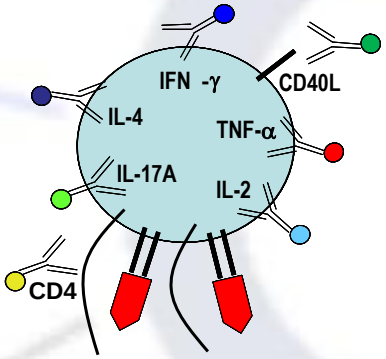
HIV contraction: 30 out of 185 vaccinees included in the analysis

COMPASS versus Polyfunctionality after adjustment

Predictive model of HIV contraction:
Ex vivo polyfunctional analysis of HIV-gag specific $CD4^+$ T cells post vaccination (IFN-γ, IL-4, IL-17A, CD40L, IL-2, TNF-α).

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





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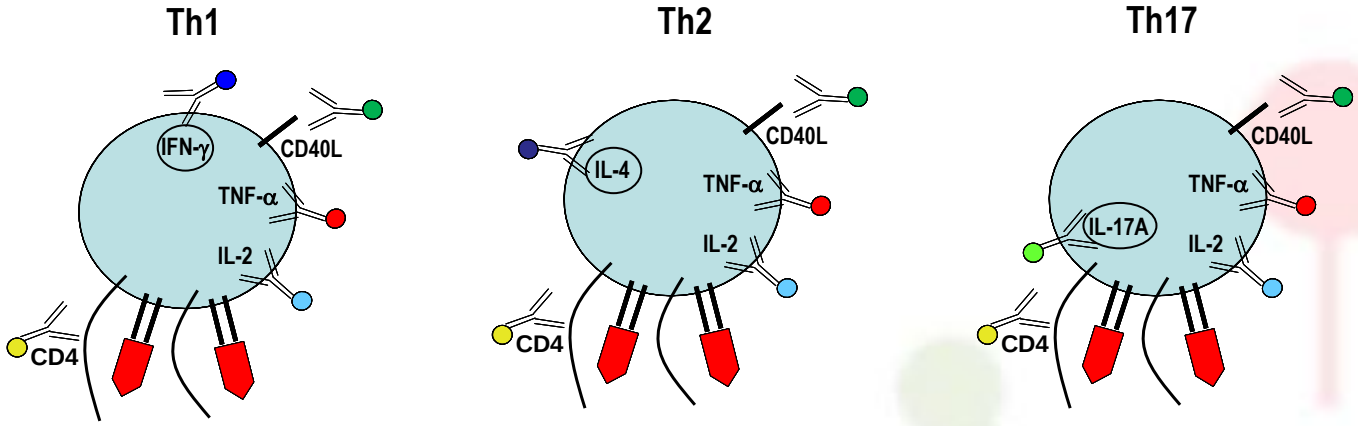
Lin *et al.* Nat Biotech 2015, Sauce *et al.* Sci Rep 2016

CD4+ T cell polyfunctionality and HIV vaccine protection

RV144 HIV case-control clinical trial:



Sauce *et al.* Sci Rep 2016

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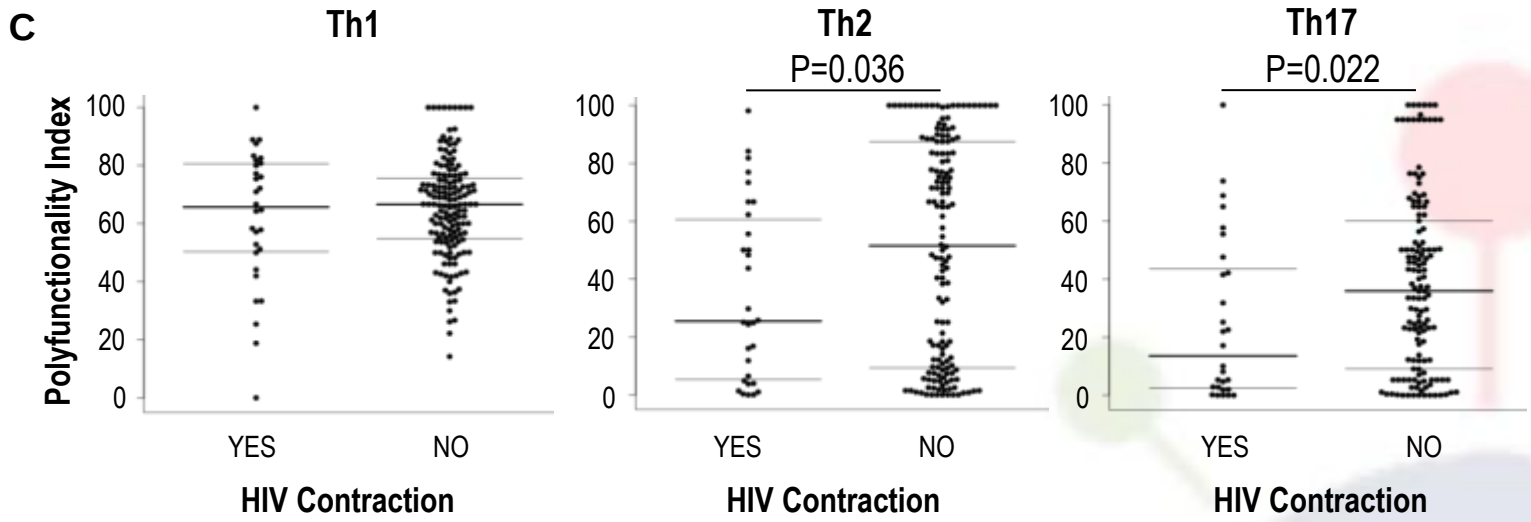

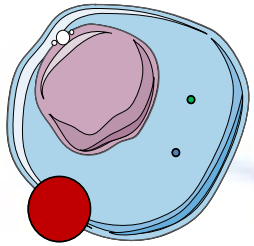

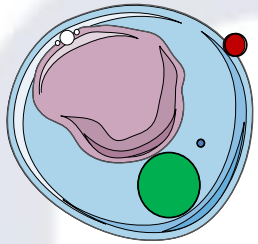

CD40L
IL-2
TNF-α

	q	φ_{CD40L}	φ_{IL2}	φ_{TNFa}
Th1	0	0	1000	0
Th2	0	1000	0	0
Th17	0	20	375	0

Sauce *et al.* Sci Rep 2016

CD4+ T cell polyfunctionality and HIV vaccine protection

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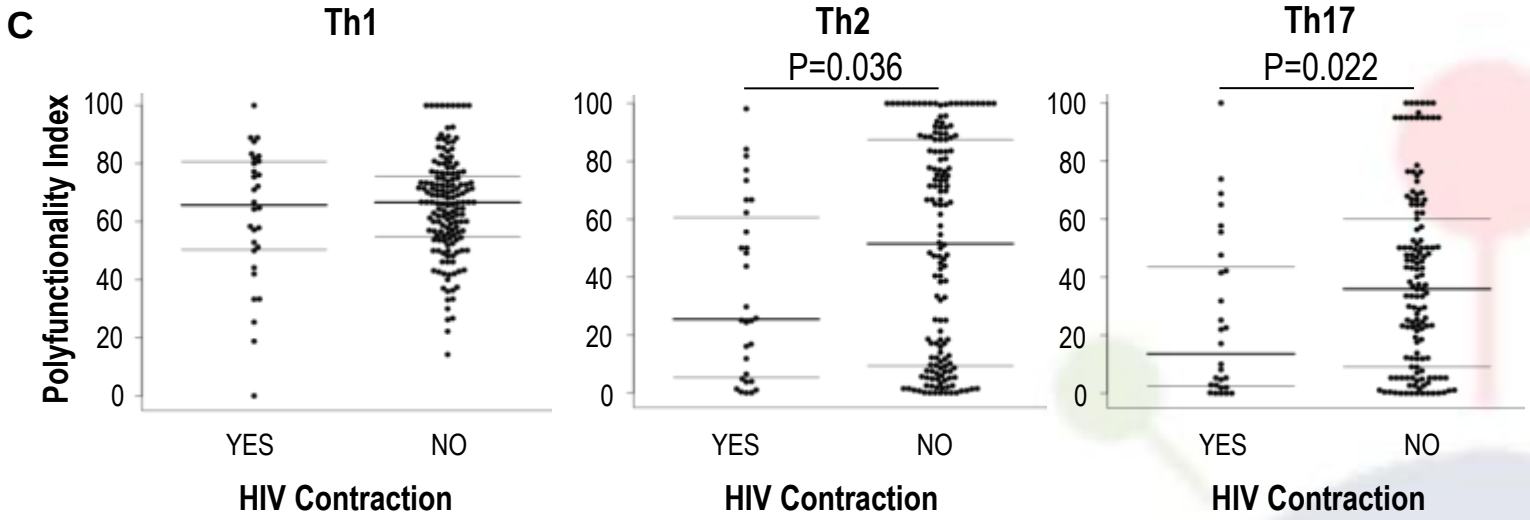
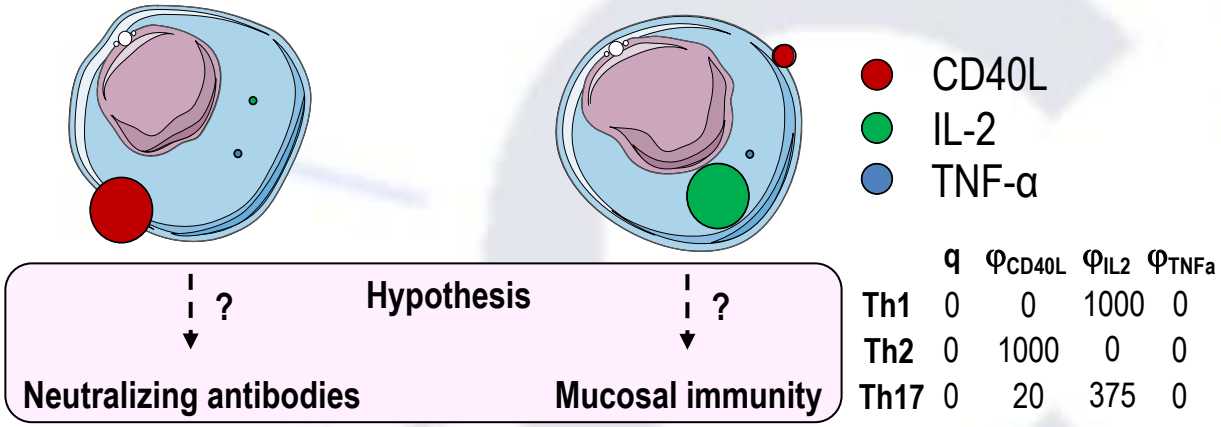
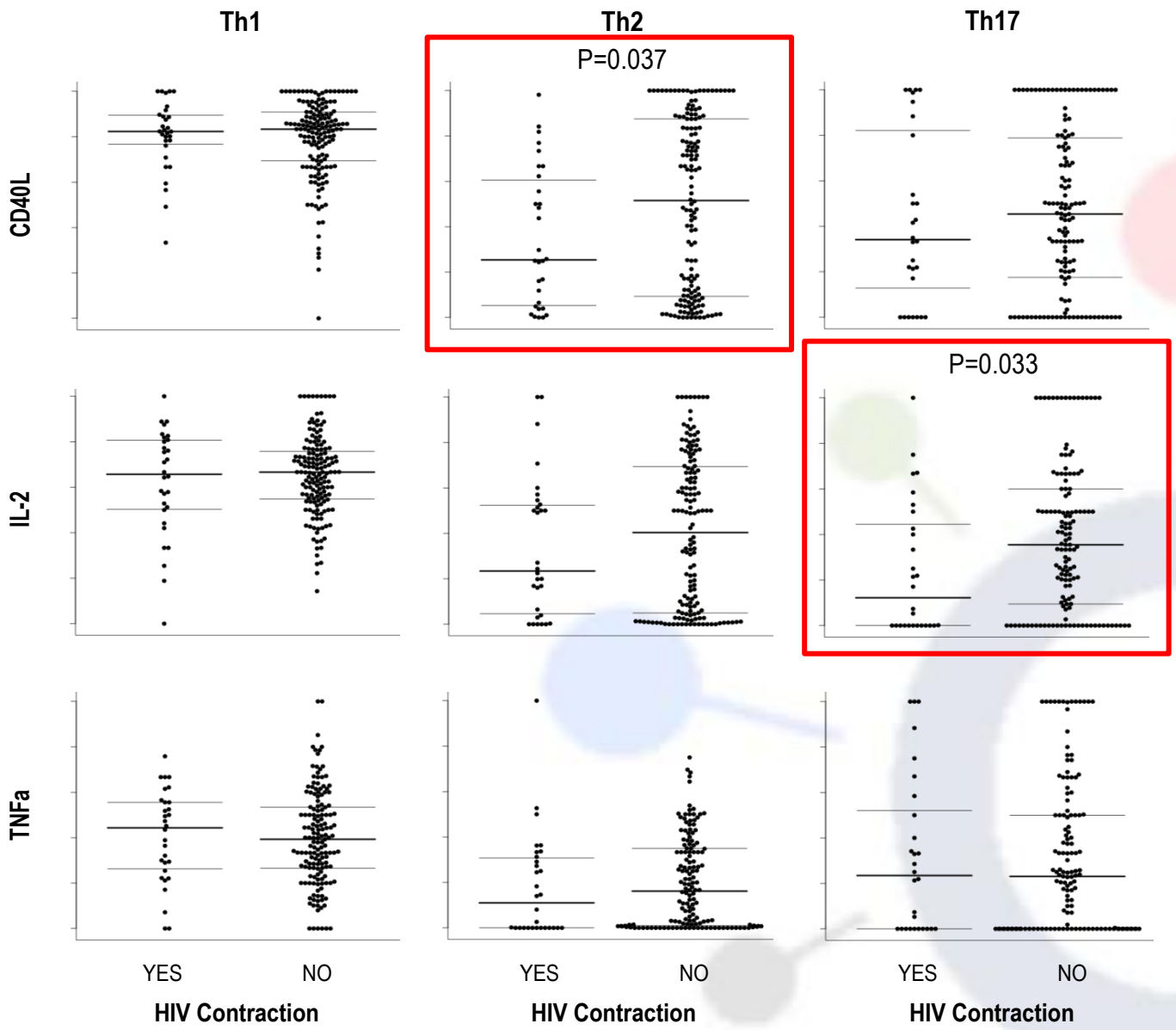




	q	φ_{CD40L}	φ_{IL2}	φ_{TNFa}
Th1	0	0	1000	0
Th2	0	1000	0	0
Th17	0	20	375	0

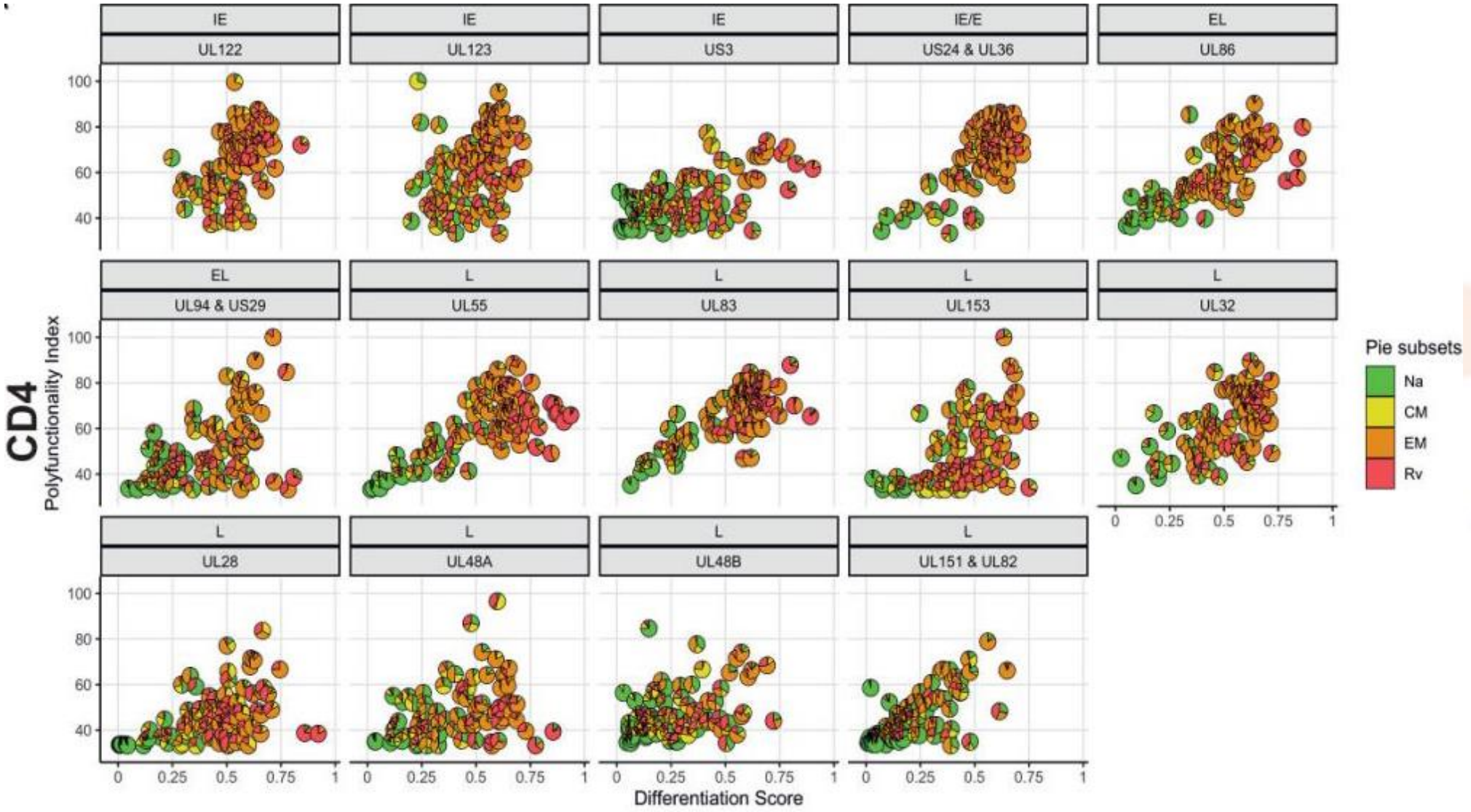
Sauce *et al.* Sci Rep 2016

CD4+ T cell polyfunctionality and HIV vaccine protection



Sauce *et al.* Sci Rep 2016

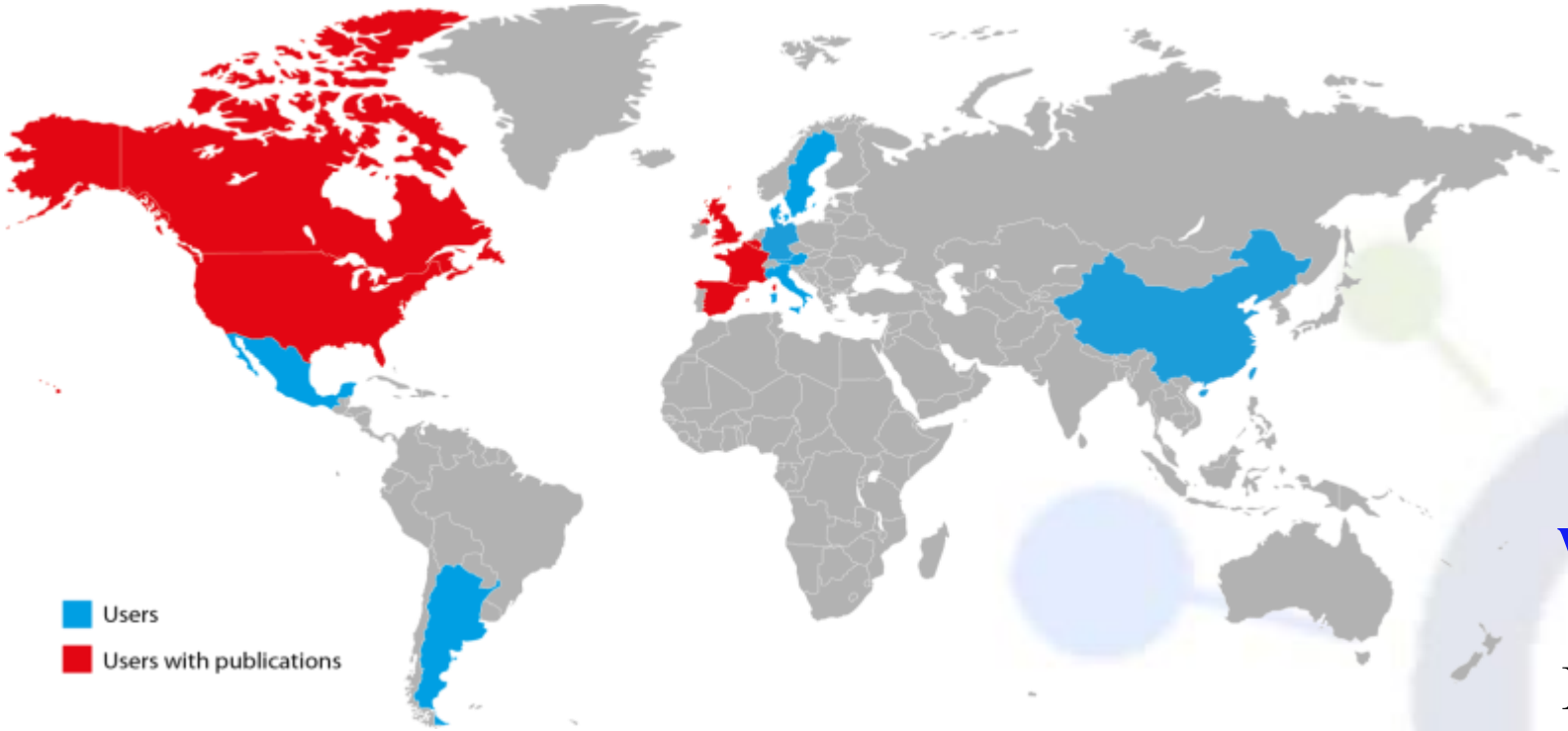
CMV-specific T cell differentiation and function



CD4 T cell differentation and polyfunctionality are positively correlated.

Reus *et al.* Front Immunol 2021

Funky Cells Tool Box software



Funky Cells

www.FunkyCells.com

Funky Cells Tool Box