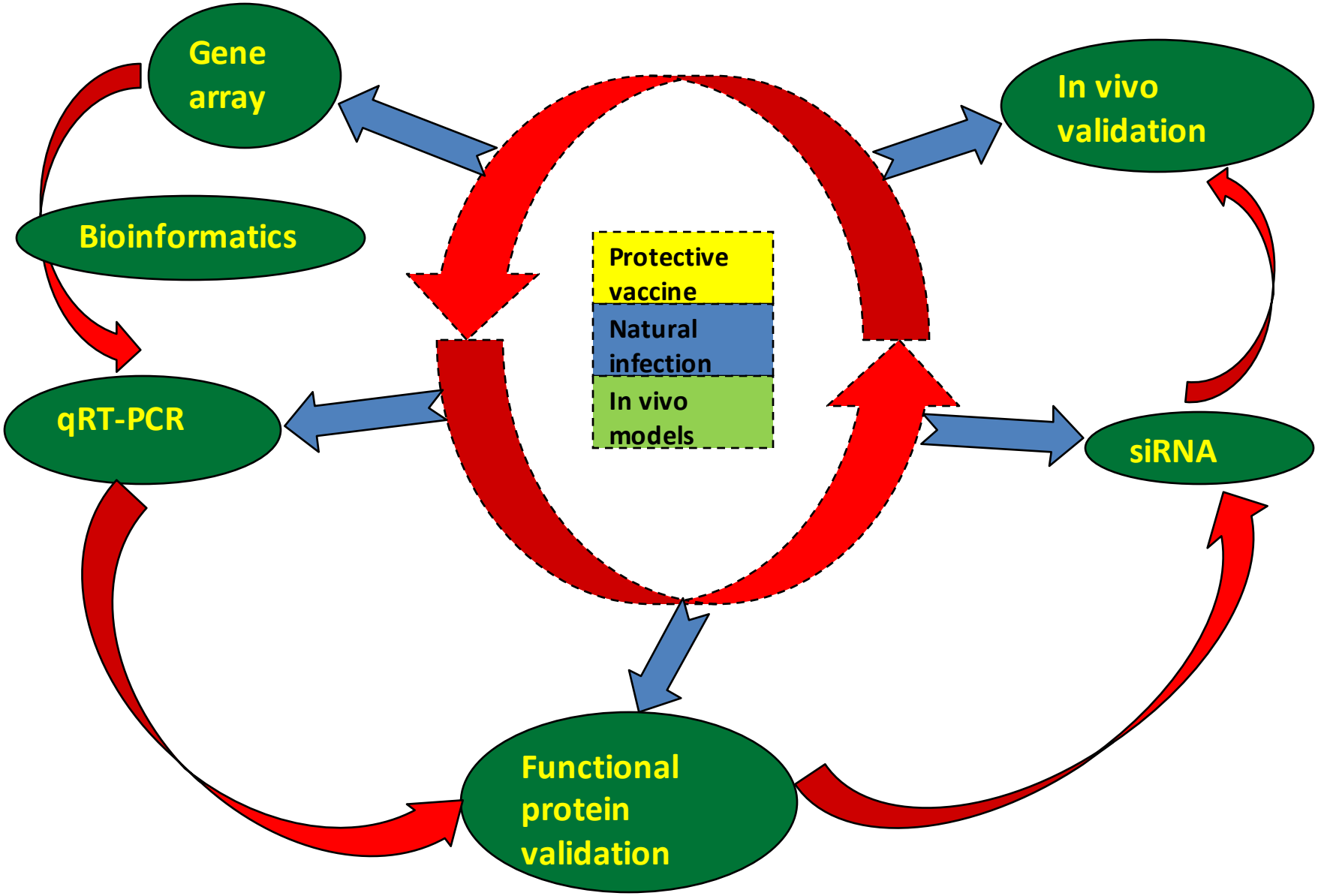
SYSTEMS BIOLOGY APPROACHES TO CORRELATES OF PROTECTION

ISBTc-FDA-NCI

Workshop on Prognostic and predictive Immunologic Markers in Cancer

System Biology platform to identify Protective immune signatures



SYSTEMS BIOLOGY AND VACCINES

- We (Pulendran , Sekaly, Douek) are using systems biology and genomics to identify :
  - the pathways (effector cells and molecules) involved in modulation of the immune response
  - the pathways (effector cells and molecules) involved in the development of memory T cells
  - the emerging immune response to a protective vaccine the yellow fever vaccine
  - The correlates of protection to vaccines , the yellow fever vaccine
  - Differences in innate immune responses elicited by viral vectors and adjuvants used in vaccines

Specific Objectives

- System Biology to identify network of pathways elicited in protective immune responses
  - Protective vaccines (YF, flu, Hep A and other adult vaccines)
  - Natural protection (Elite HIV controllers, HCV controllers)
  - Chronic infection (HIV, HCV and TB)
  - Whole blood
  - Memory T cell and B cell subsets
  - Ag specific T cells (profile as low as 1000 cells)
    - Tetramer sorting
    - Single peptide stimulation
  - Innate immune genes
    - Innate immune signature of dendritic cells infected with viral vaccine vectors [Poxvirus (MVA, NYVAC), Adeno viruses, YF, HIV] or triggered with TLR ligands

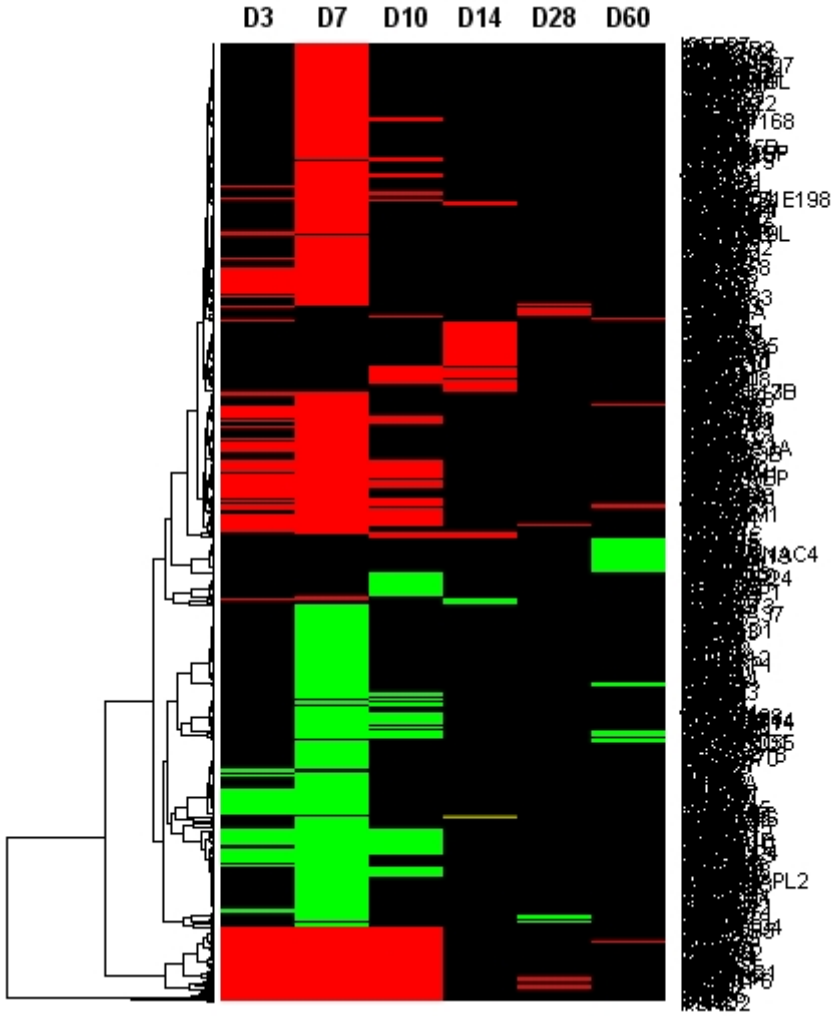
STRATEGY

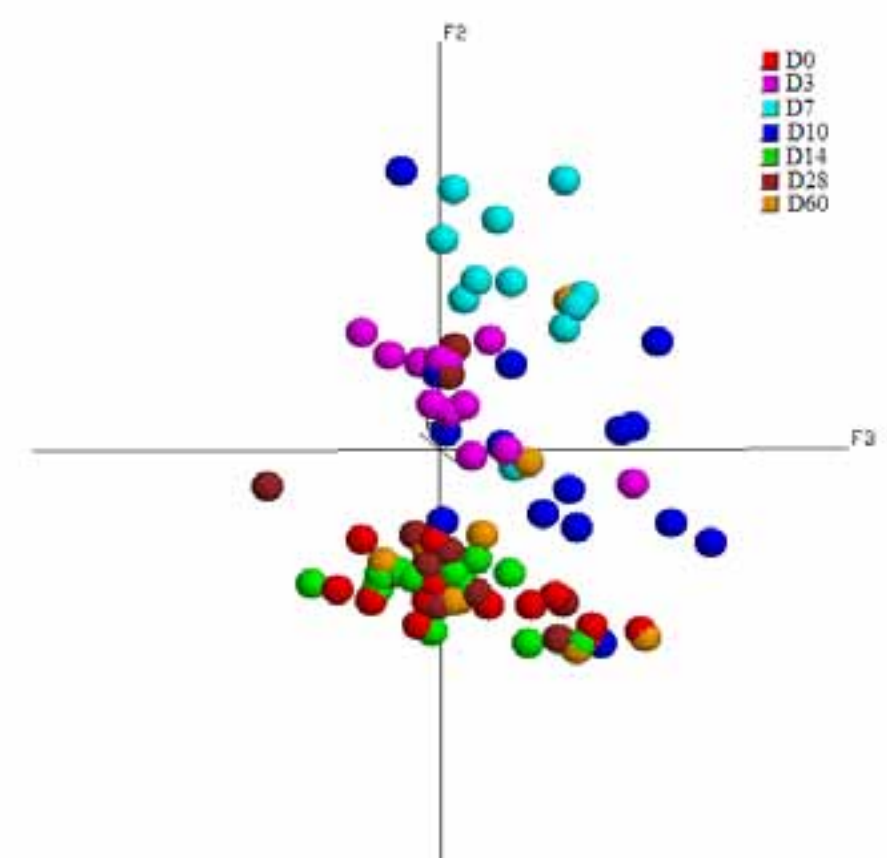
- Work with very homogeneous groups of subjects : gender , ethnic group , age , disease status
- Work with very homogeneous populations of cells
- Work with minimal number of cells
- Develop assays which are multi-parametric , high throughput and highly sensitive
- Multiple validation steps including PCR , proteomics and genome wide siRNAs
- Develop integrated data base to help identify immune correlates of protection and of disease progression

Identification of Gene expression signatures of Immune mediated correlates of protection

The Yellow fever study

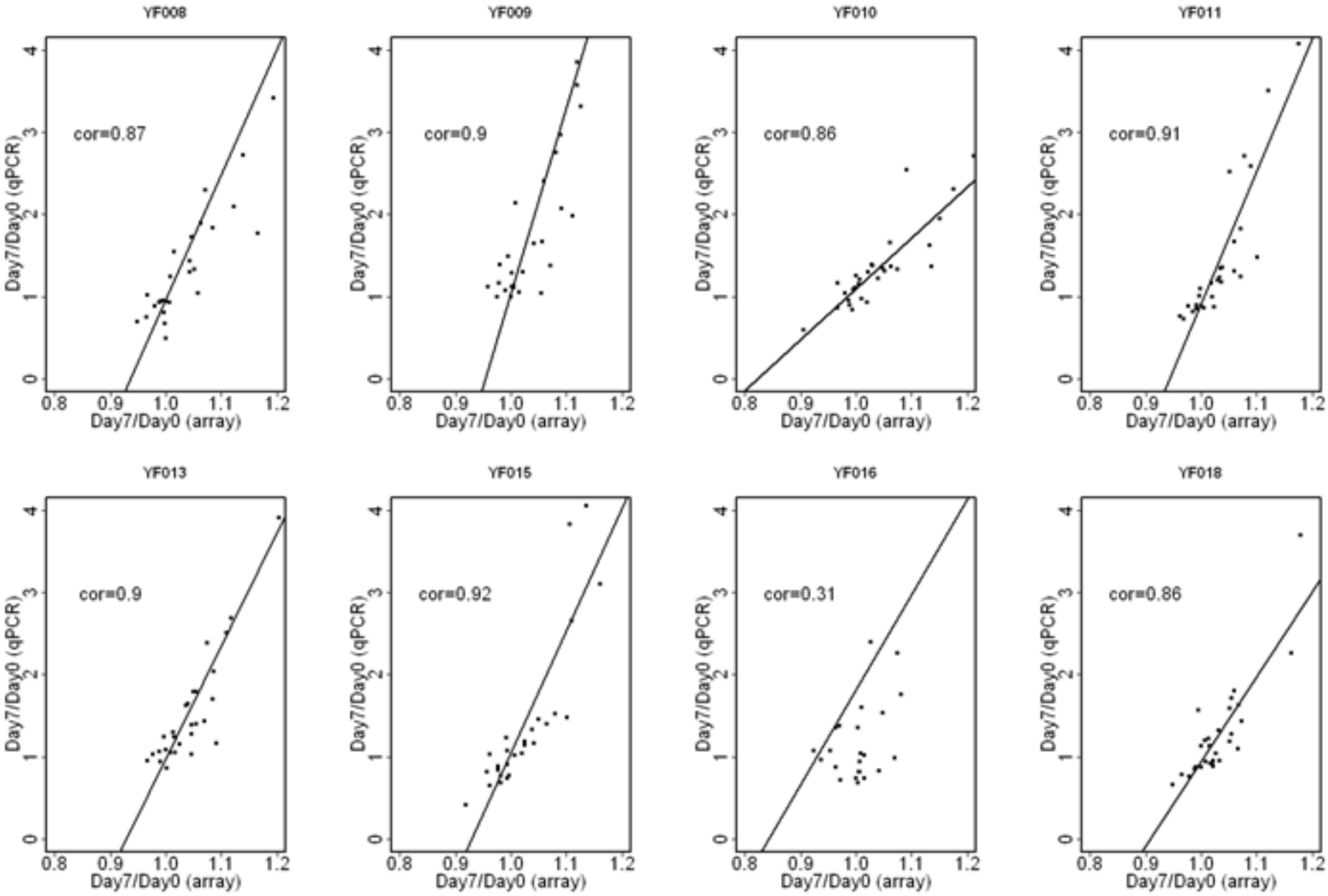
Vaccination with YF17D induces and early gene modulation in PBMCs

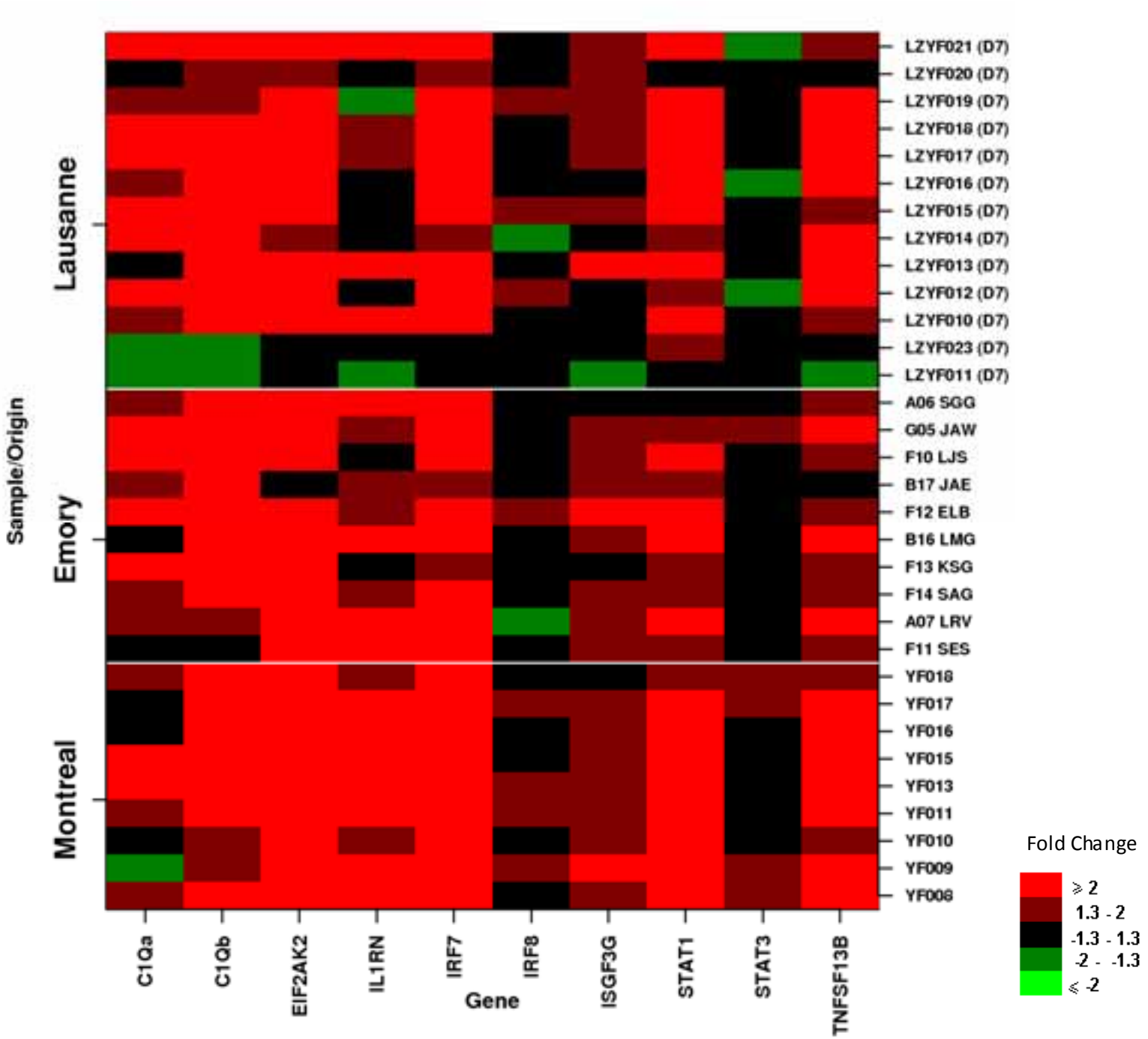




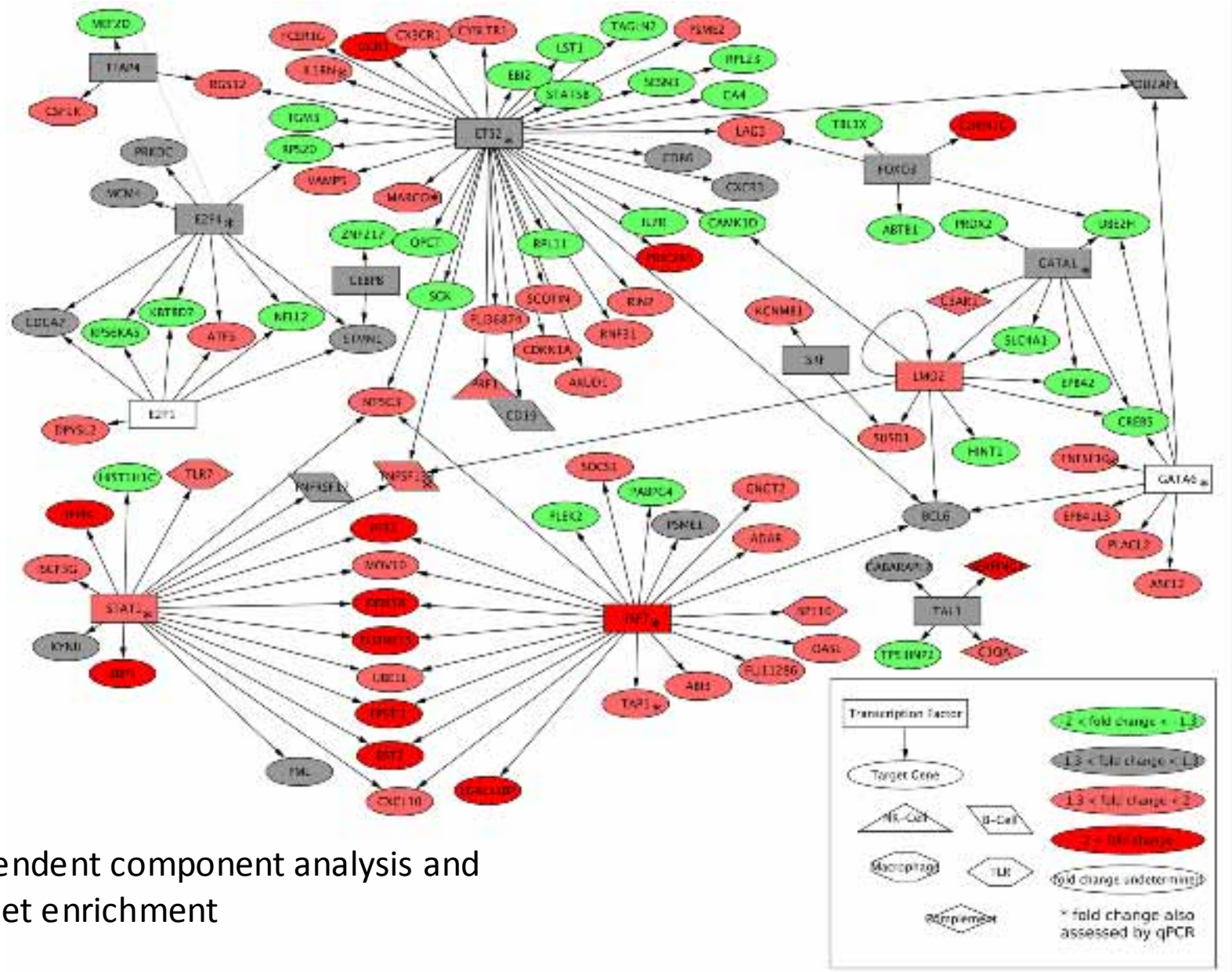
594 significantly modulated genes

Validation of gene expression signatures : strong correlation between PCR and Illumin




Sample/Origin
Lausanne
Emory
Montreal
LZYF021 (D7)
LZYF020 (D7)
LZYF019 (D7)
LZYF018 (D7)
LZYF017 (D7)
LZYF016 (D7)
LZYF015 (D7)
LZYF014 (D7)
LZYF013 (D7)
LZYF012 (D7)
LZYF010 (D7)
LZYF023 (D7)
LZYF011 (D7)
A06 SGG
G05 JAW
F10 LJS
B17 JAE
F12 ELB
B16 LMG
F13 KSG
F14 SAG
A07 LRV
F11 SES
YF018
YF017
YF016
YF015
YF013
YF011
YF010
YF009
YF008
C1Qa
C1Qb
EIF2AK2
IL1RN
IRF7
IRF8
ISGF3G
STAT1
STAT3
TNFSF13B
Gene
Fold Change
⩾ 2
1.3 - 2
-1.3 - 1.3
-2 - -1.3
⩽ -2

Transcriptional network of differentially expressed genes after YF17D vaccination (inferred by gene set enrichment)

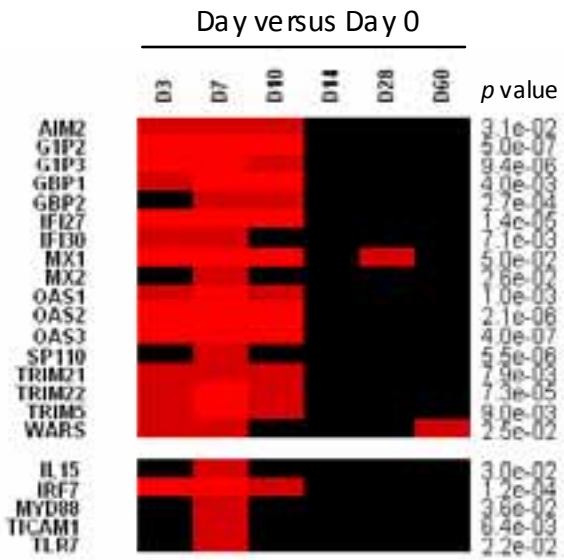


Independent component analysis and gene set enrichment

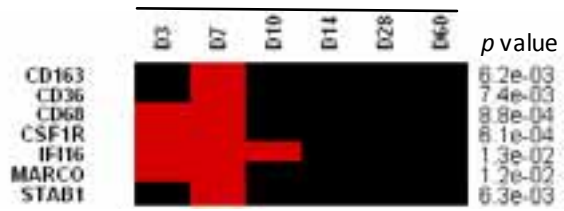
The YF Vaccine induces a multicellular innate immune response

YF vaccine induces the expression of genes of multiple cell lineages

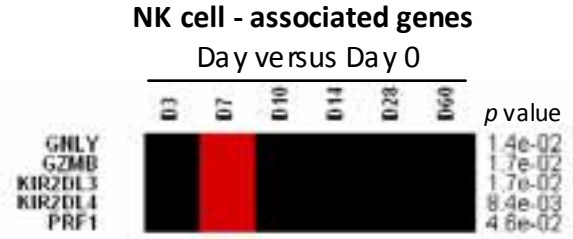
Interferon - induced and TLR - associated genes



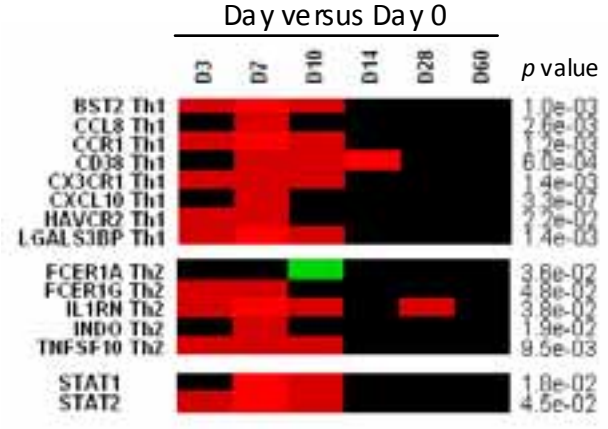
Macrophage - associated genes



NK cell - associated genes

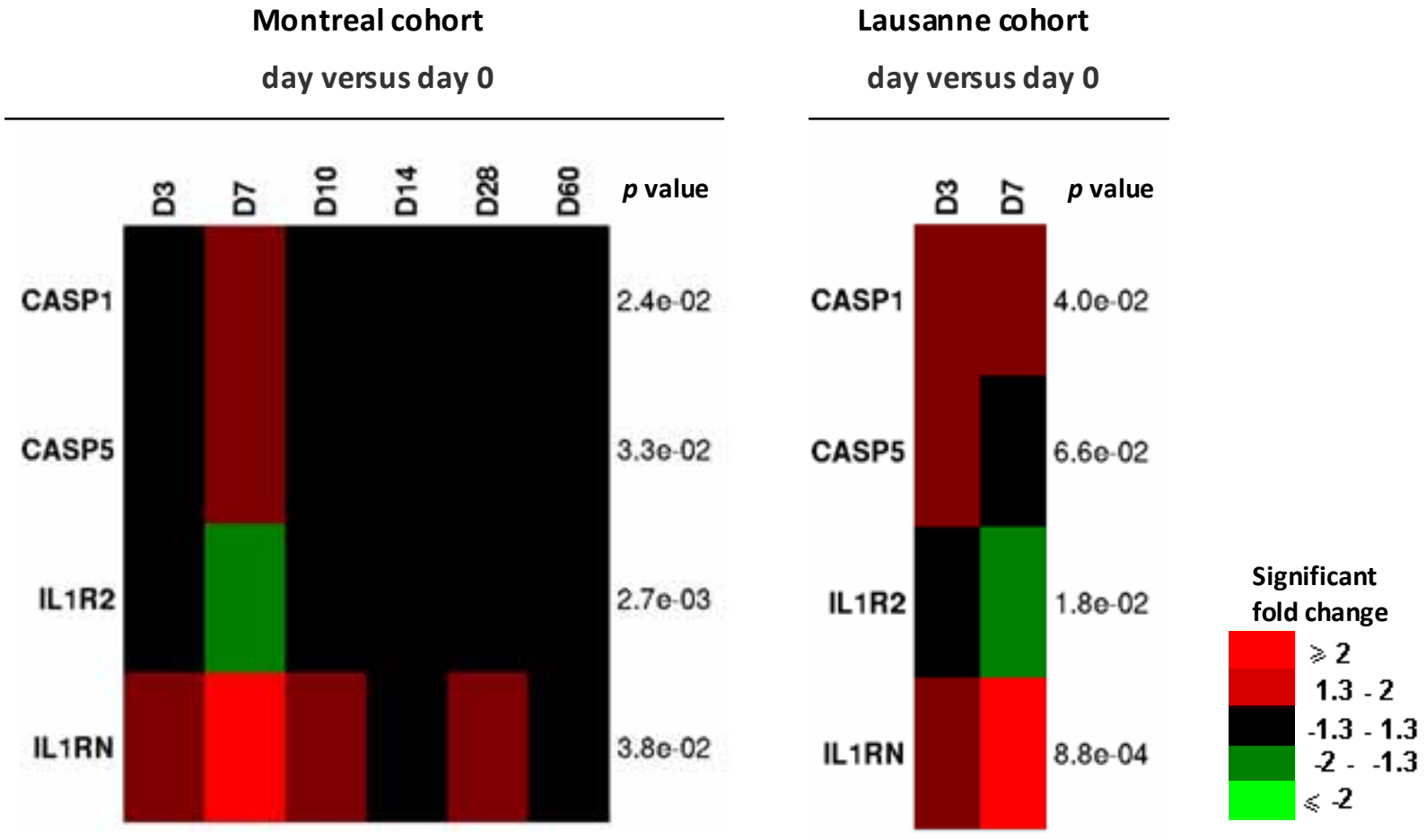


Th1/Th2 - associated genes

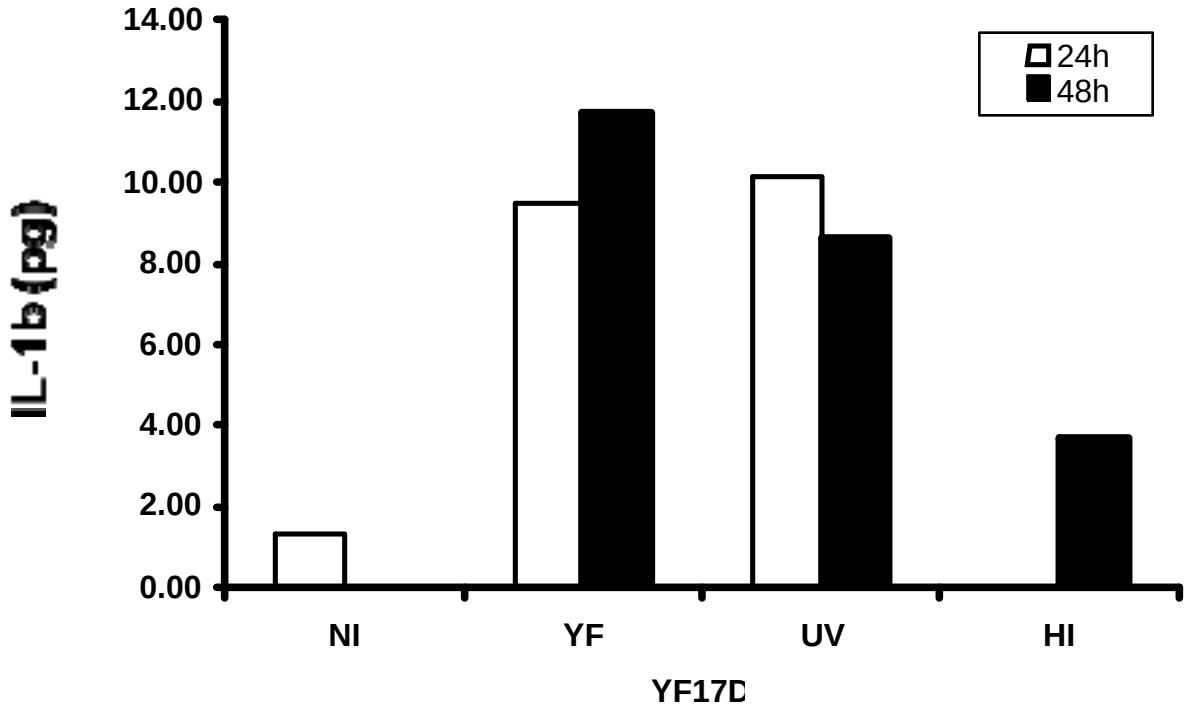


Complement - associated genes

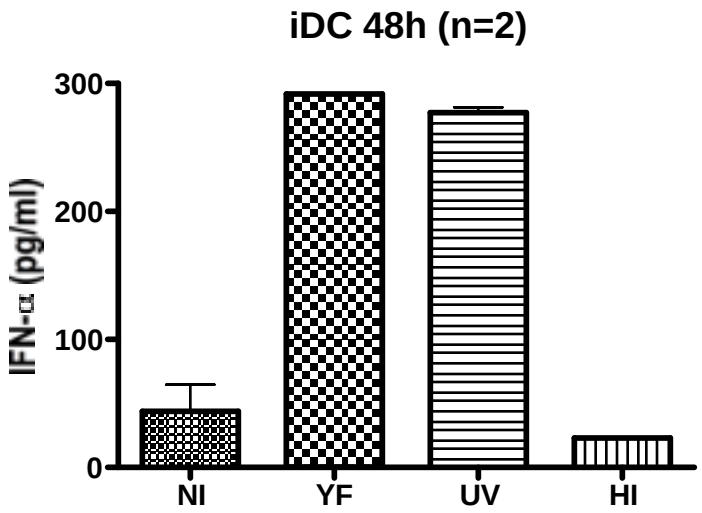
YF17D vaccination induces the expression of genes associated with inflammasome assembly

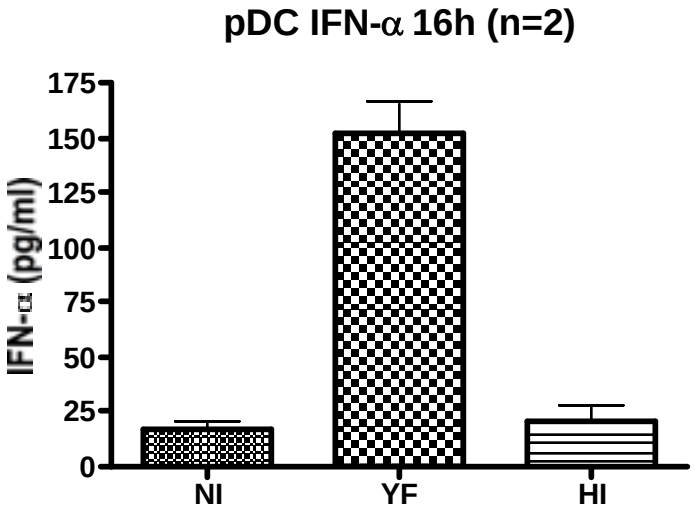


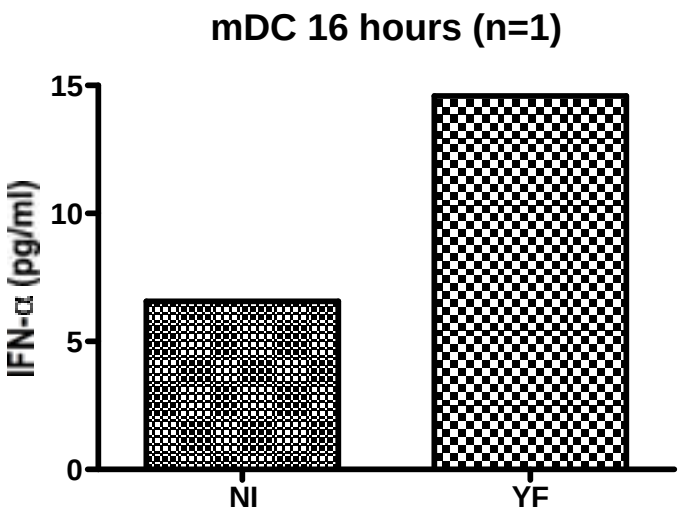
YF17D virus stimulates IL-1ß secretion by iDCs *in vitro*



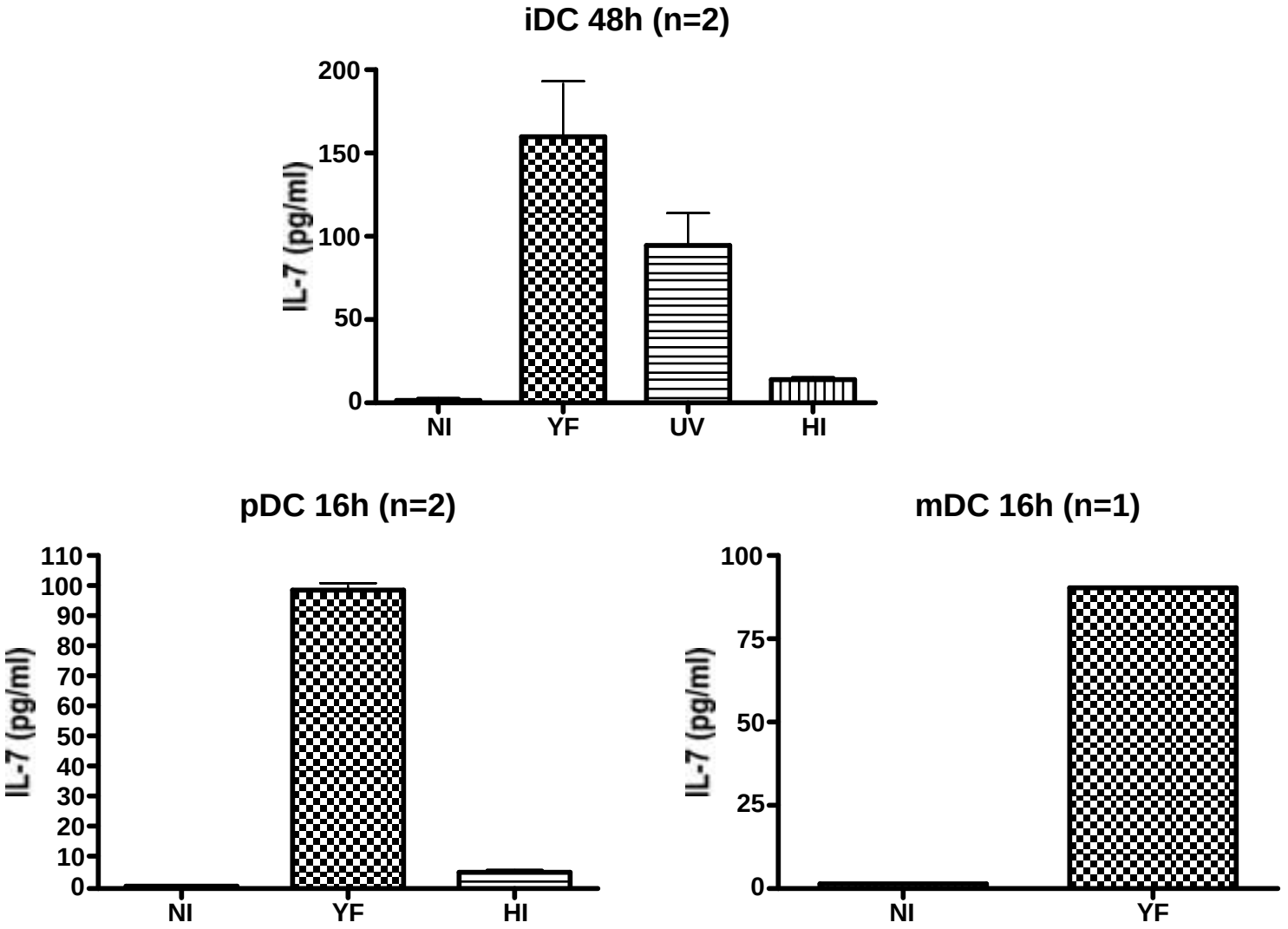
YF17D virus stimulates IFN-a secretion by DCs *in vitro*







YF17D virus stimulates IL-7 secretion by DCs *in vitro*



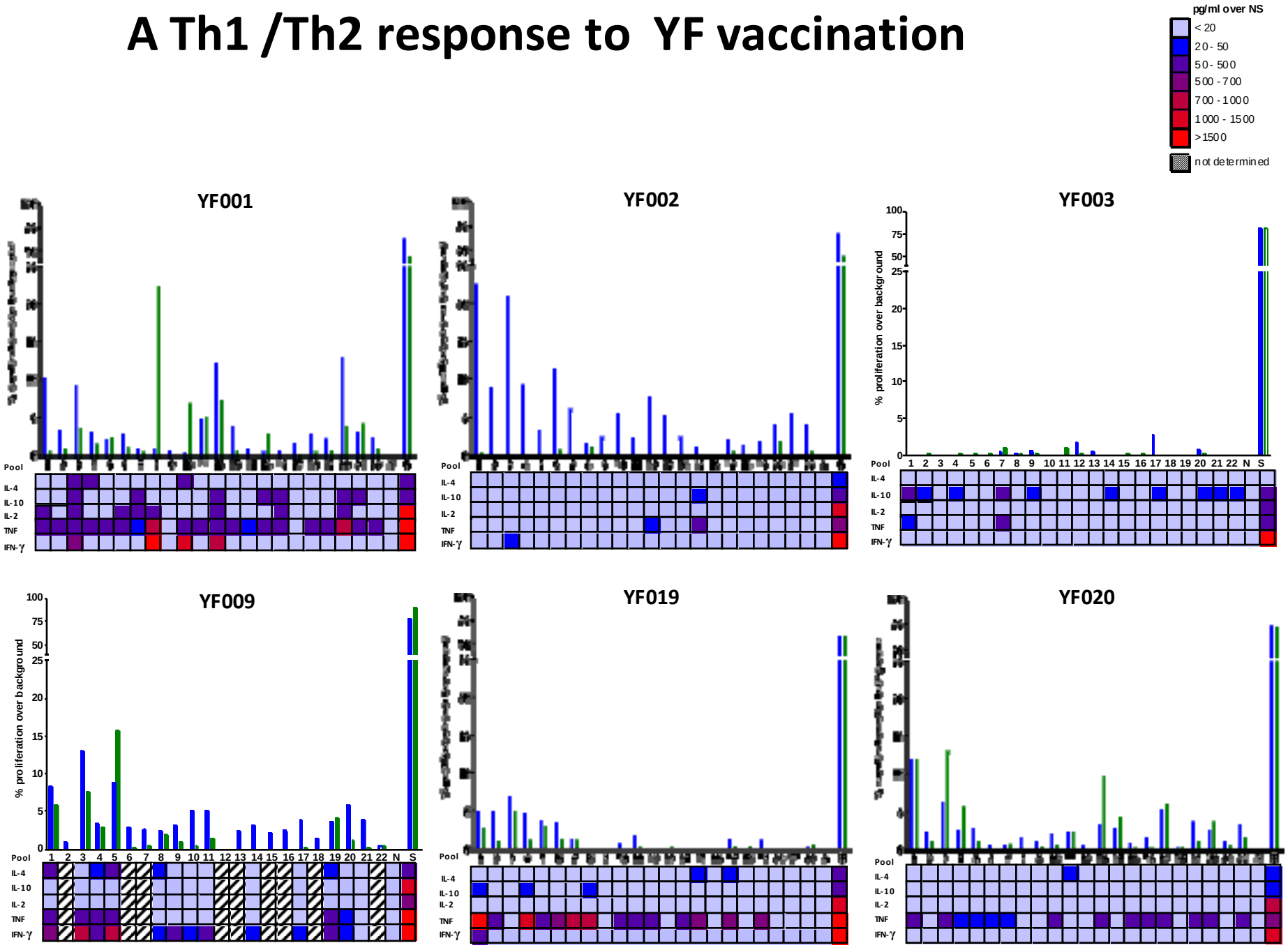
YF17D virus stimulates the modulation of transcription factors and antigen-processing genes in DCs

Fold change	Gene	Annotation
3.44	IRF7	Homo sapiens interferon regulatory factor 7 (IRF7), transcript variant b, mRNA.
2.99	IRF1	Homo sapiens interferon regulatory factor 1 (IRF1), mRNA.
2.98	STAT1	Homo sapiens signal transducer and activator of transcription 1, 91kDa (STAT1), transcript variant beta, mRNA.
2.94	SOCS1	Homo sapiens suppressor of cytokine signaling 1 (SOCS1), mRNA.
2.94	EIF2AK2	Homo sapiens eukaryotic translation initiation factor 2-alpha kinase 2 (EIF2AK2), mRNA.
2.83	STAT1	Homo sapiens signal transducer and activator of transcription 1, 91kDa (STAT1), transcript variant alpha, mRNA.
2.31	STAT1	Homo sapiens signal transducer and activator of transcription 1, 91kDa (STAT1), transcript variant alpha, mRNA.
2.09	SOCS3	Homo sapiens suppressor of cytokine signaling 3 (SOCS3), mRNA.
-4.26	E2F2	Homo sapiens E2F transcription factor 2 (E2F2), mRNA.
5.03	HLA-DRB1	Homo sapiens major histocompatibility complex, class II, DR beta 1 (HLA-DRB1), mRNA.
2.33	TAP1	Homo sapiens transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) (TAP1), mRNA.
2.22	LAMP3	Homo sapiens lysosomal-associated membrane protein 3 (LAMP3), mRNA.
2.01	LRAP	Homo sapiens leukocyte-derived arginine aminopeptidase (LRAP), mRNA.

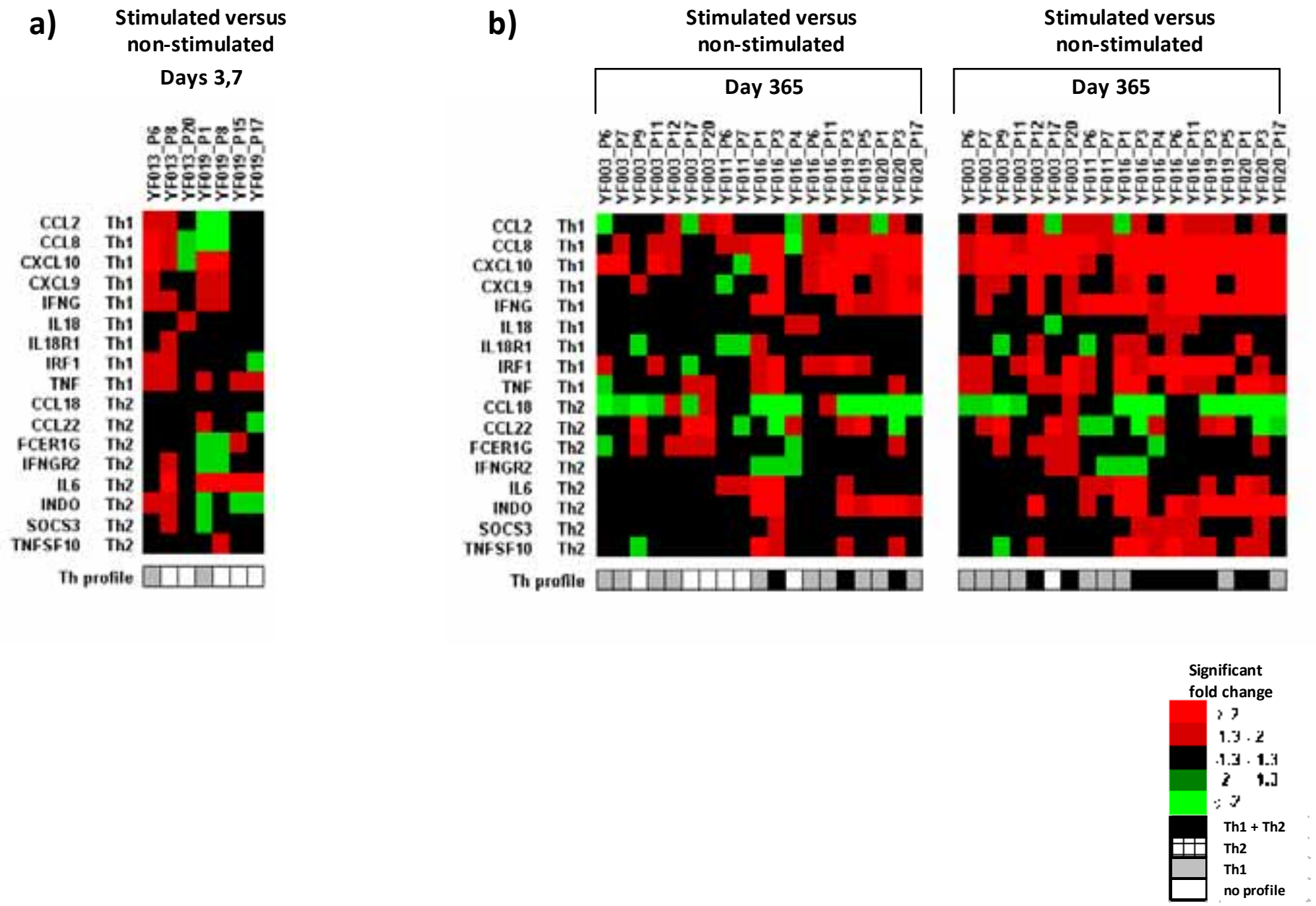
YF17FD induces a complex adaptive immune response

A Th1 /Th2 response to YF vaccination

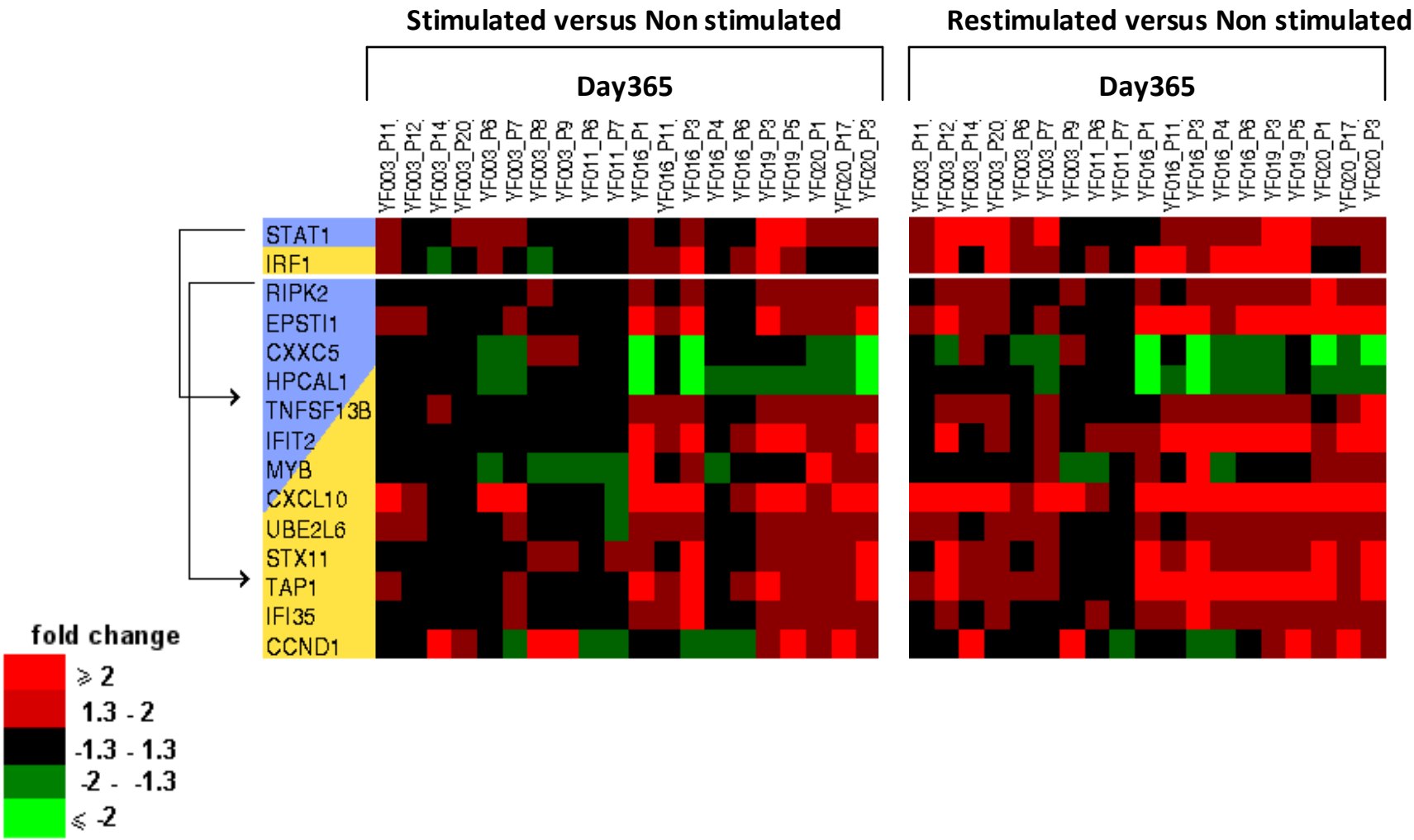
i)



Gene expression analysis of peptide specific responses is long lasting and occurs already at day 3



System Biology approach of YF-specific immune response identified major transcriptional nodes



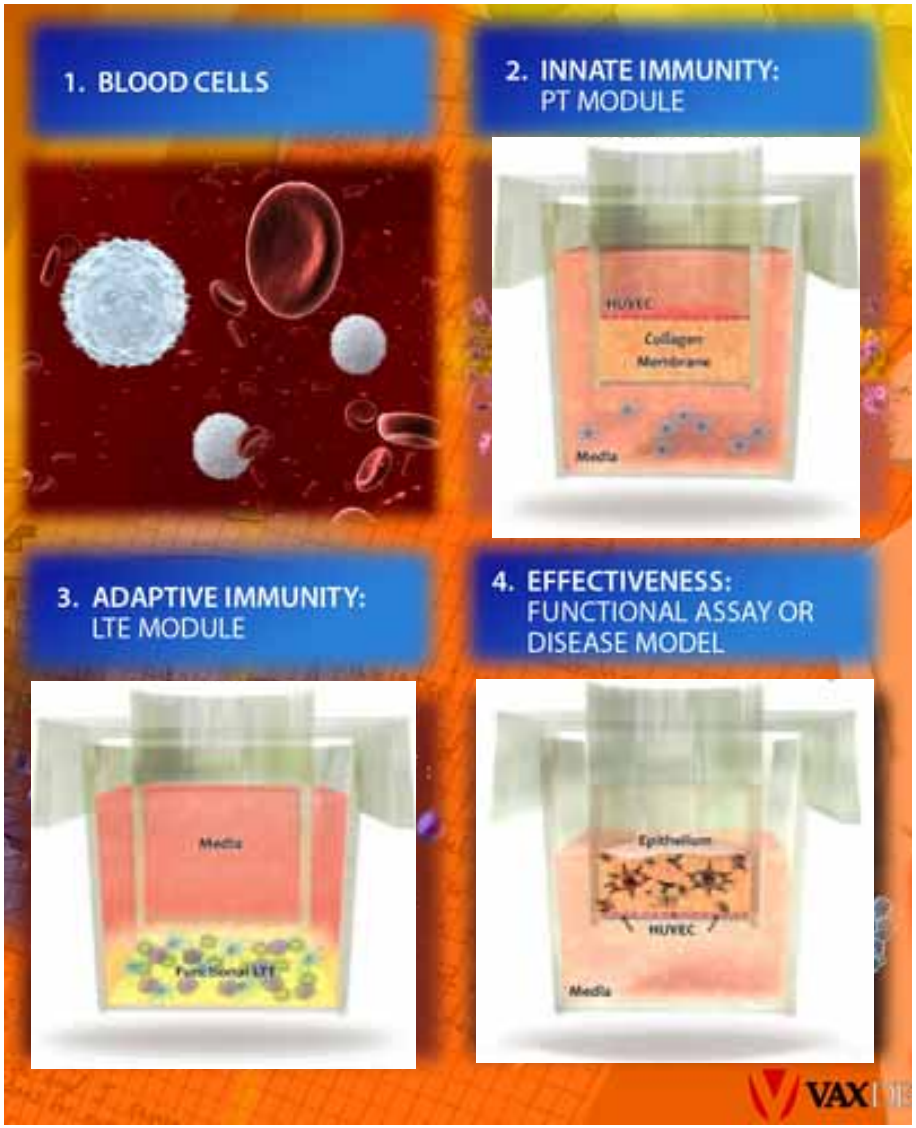
Validation of Gene expression signatures ex vivo

The MIMIC system

MIMIC™ Technology Overview

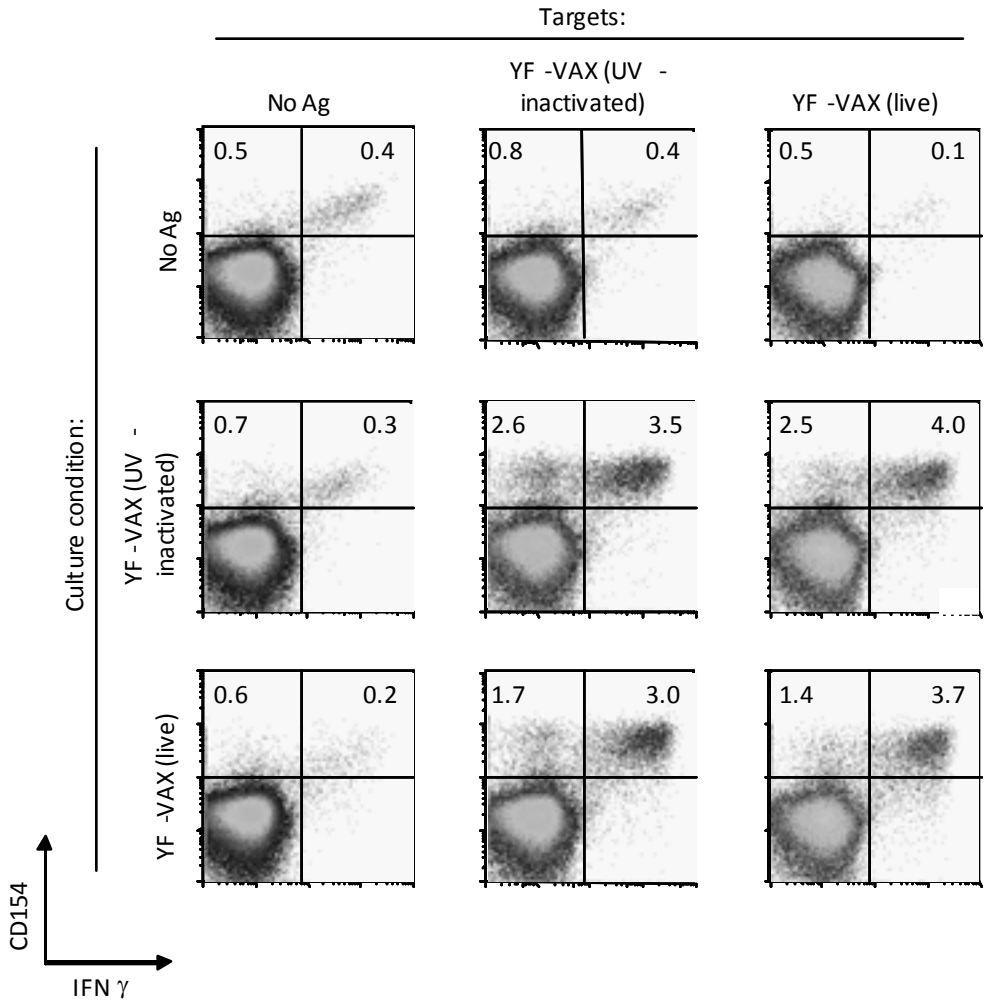
MIMIC: an *in vitro* biomimetic of the human immune response:

1. Collect leukocytes from human donors
2. Simulate innate immunity with the Peripheral Tissue (PT) Module
3. Simulate adaptive immunity with the Lymphoid Tissue Equivalent (LTE) Module
4. Measure the effectiveness of the immune response or immune modulator product



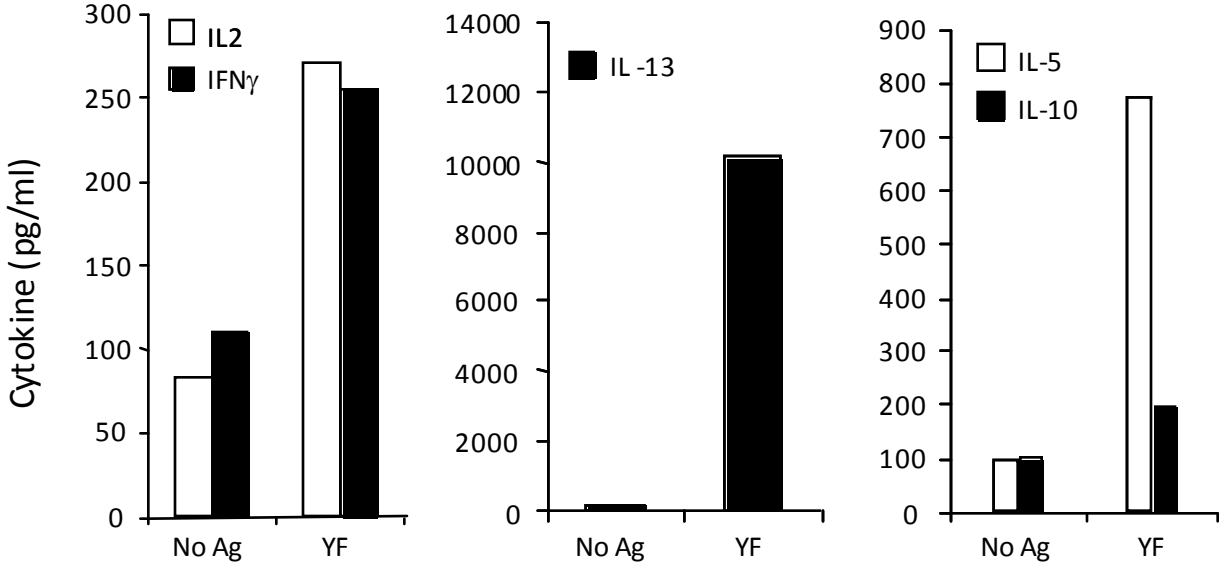
c)

Priming of naïve T cells specific for YF in the MIMIC system

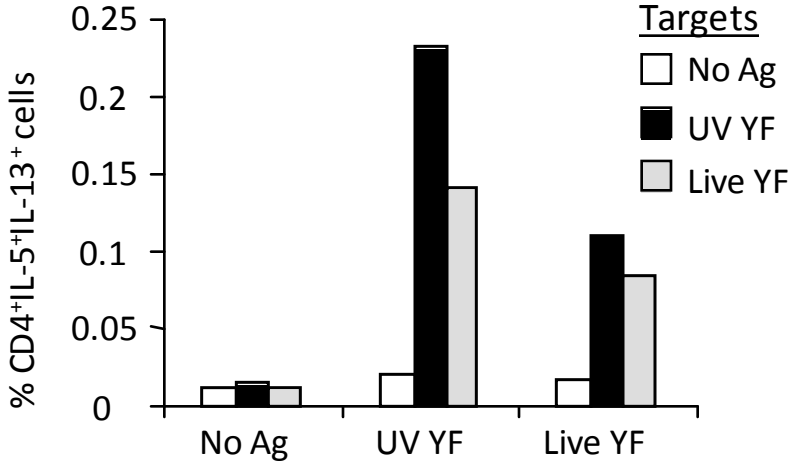


A Th1 /Th2 response to YF vaccination

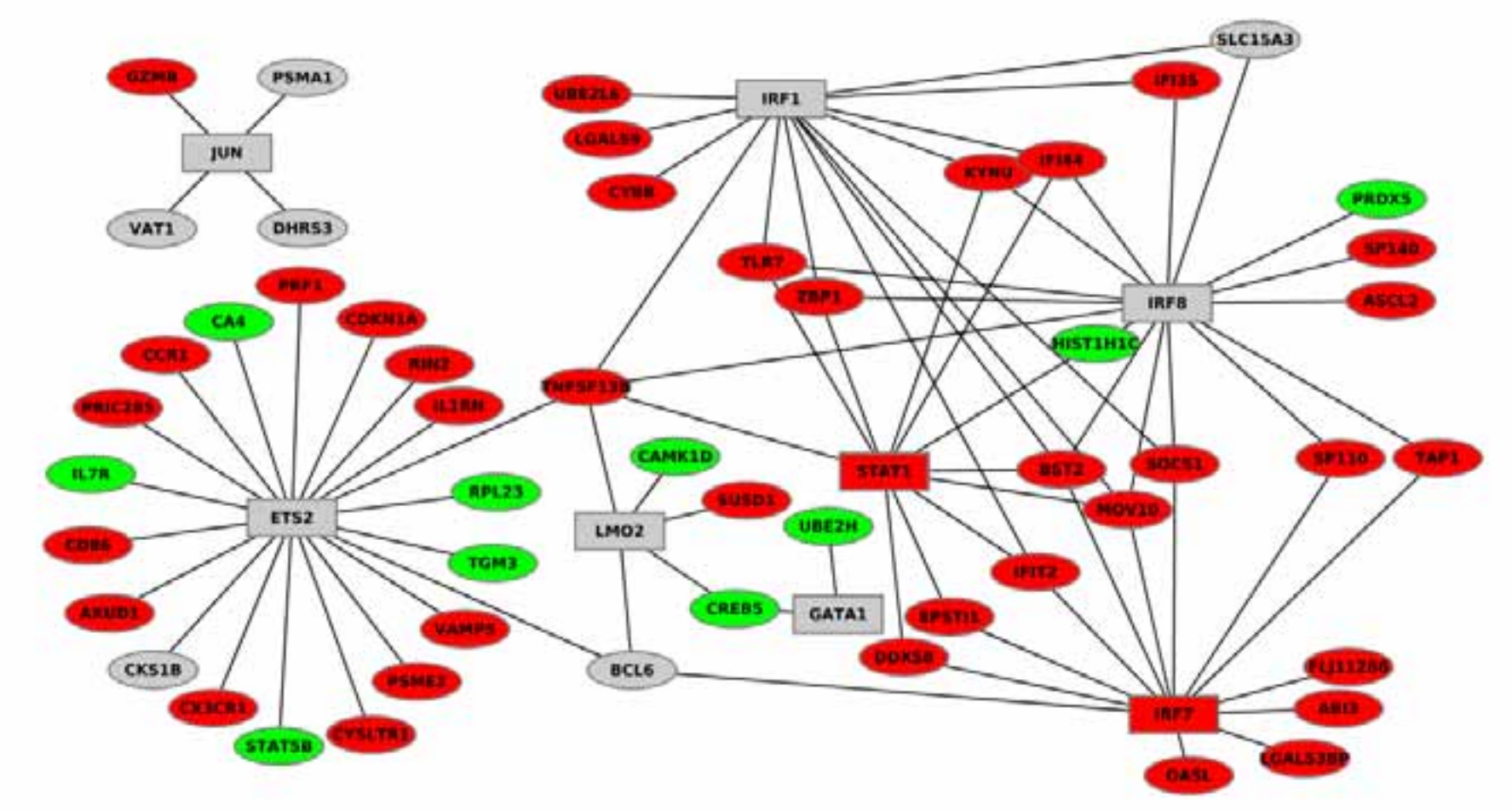
a)



b)

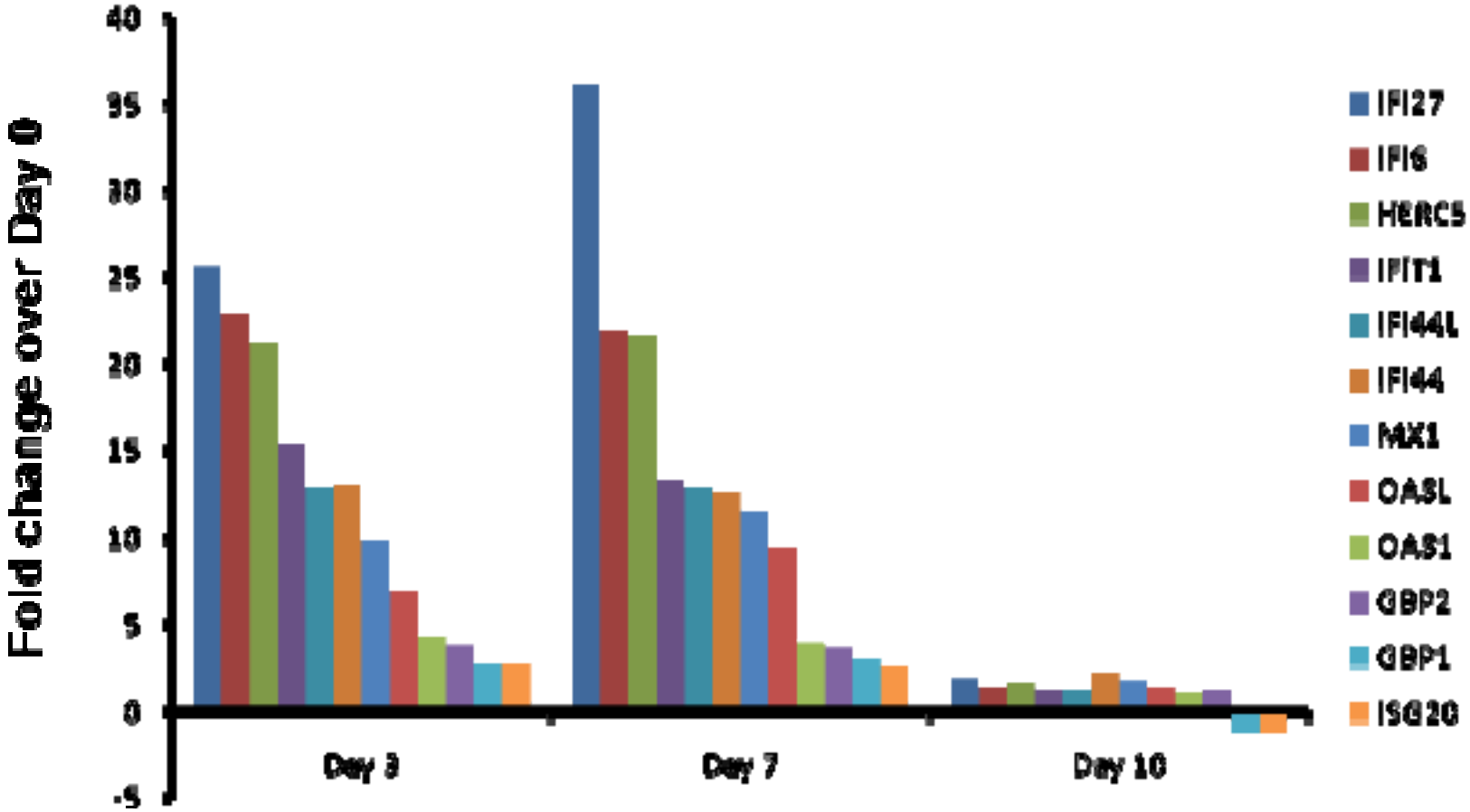


Validation of master switch genes of YF induced responses in the MIMIC systems

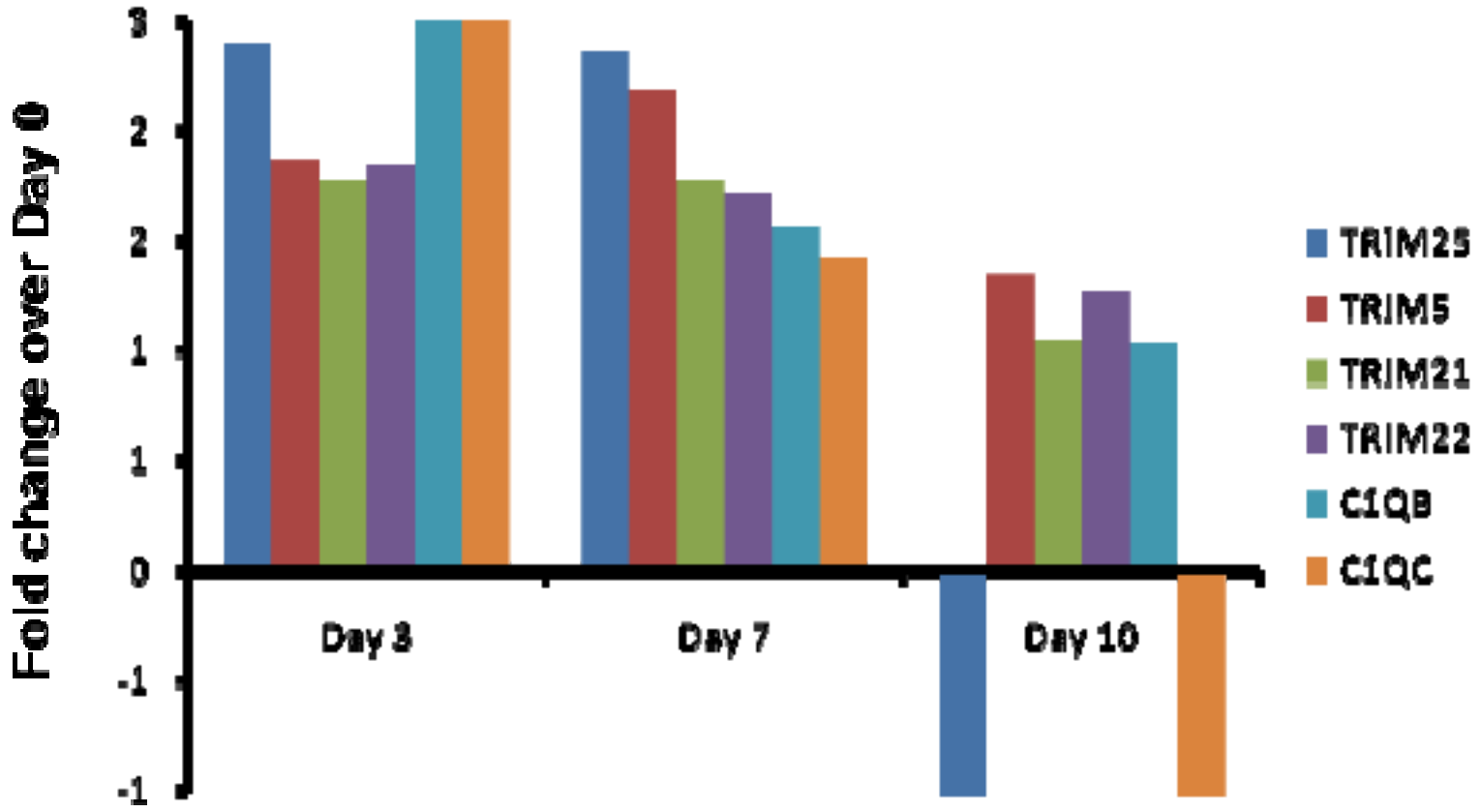


Validation of Gene expression signatures in non human primate model

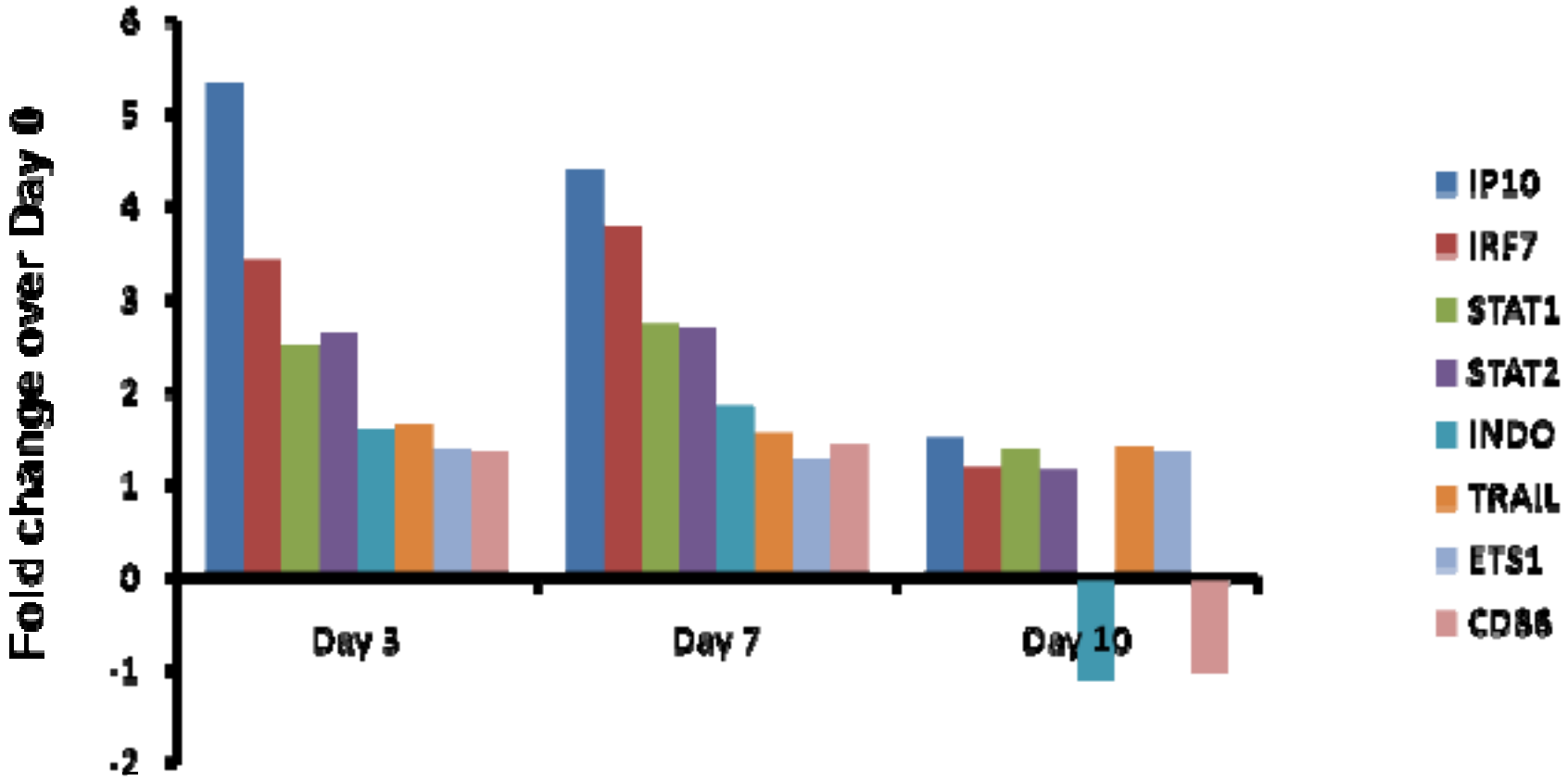
Macaques immunized with YF vaccine showed similar kinetics and transcriptional profiles to those induced in human



YF vaccine induces the expression of anti-viral genes in macaques



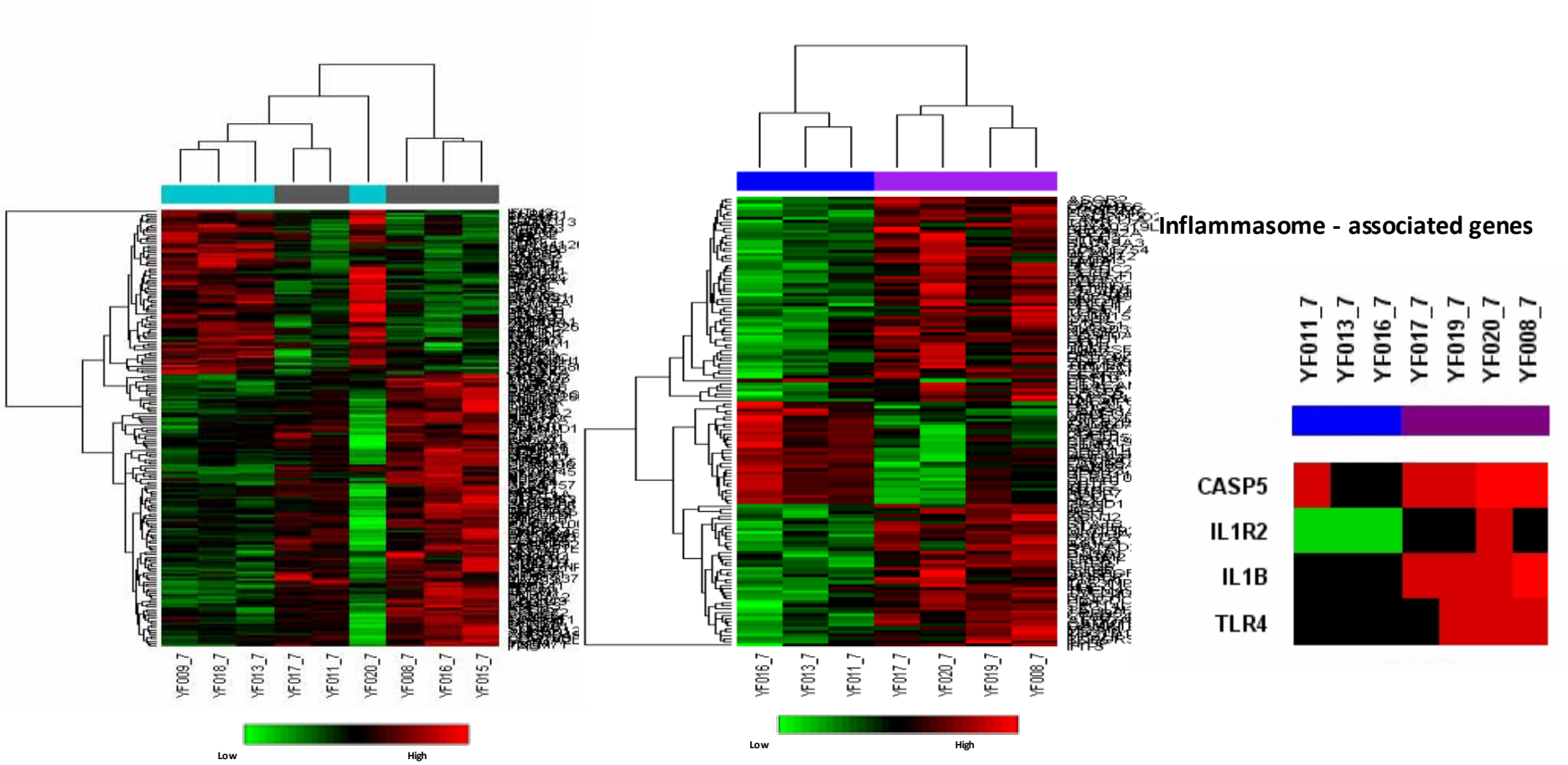
YF vaccine induces the expression of innate and adaptive immune genes in macaques



The YF vaccine response

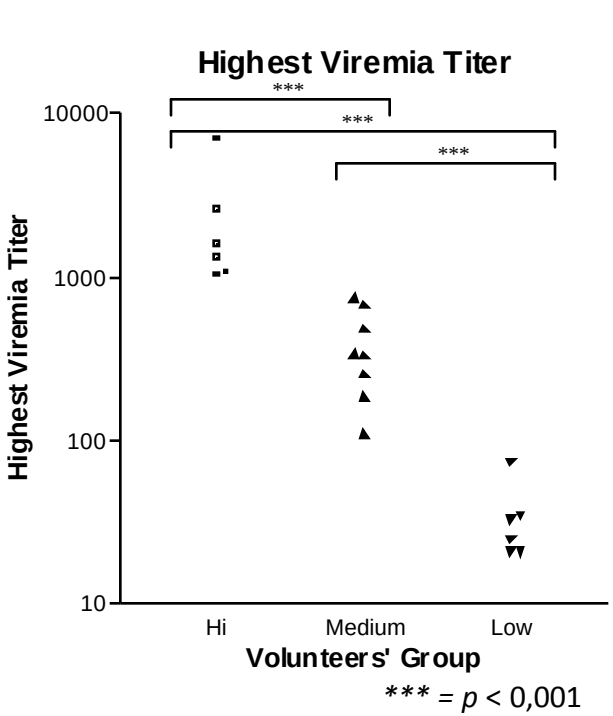
Correlates of protection

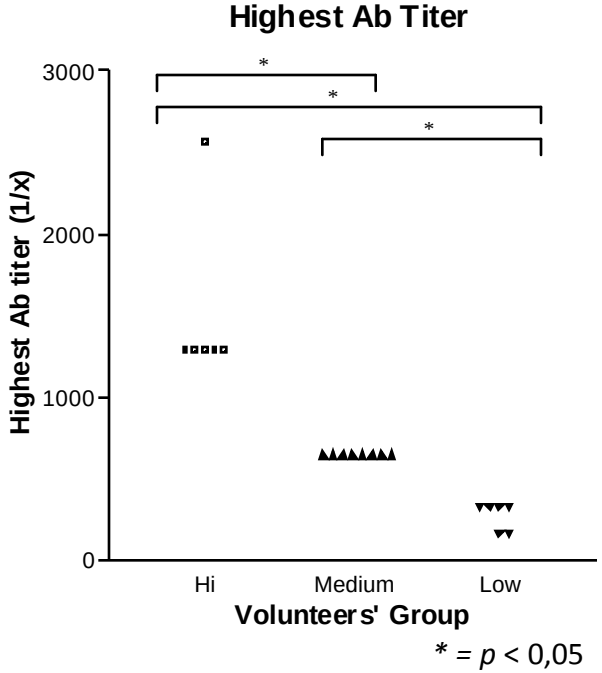
Gene signatures could predict strong CD4 response and control of viremia

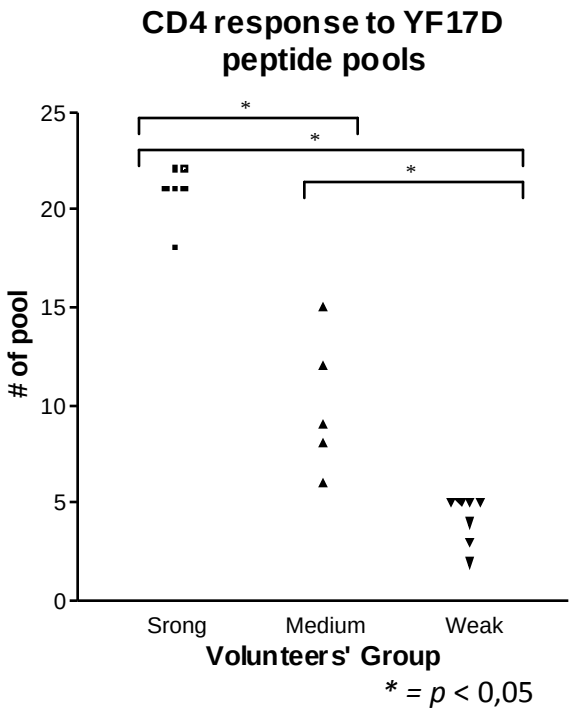


3.

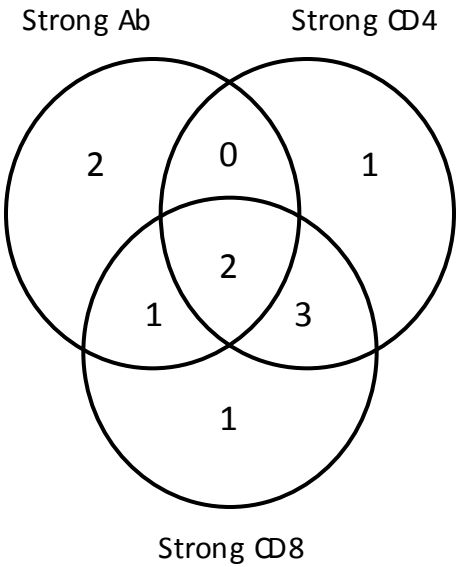
Is there a single correlate of protection

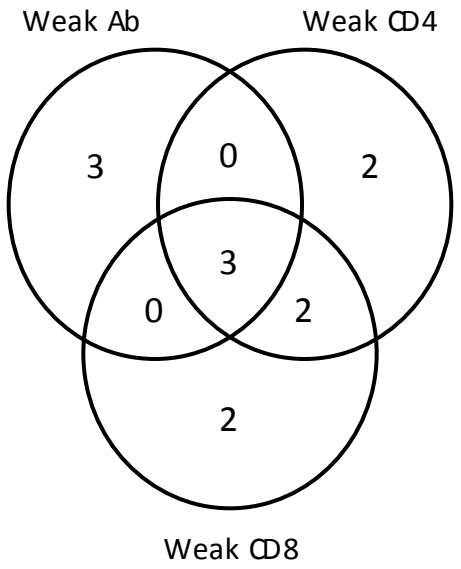






Strong humoral and cellular responses do not correlate





The Yellow fever vaccine response

- The very early induction (3 to 7 days) by the vaccine of a network of ten transcription factors precedes and drives the development of highly integrated innate and adaptive immune responses

- The protective immune response to Yellow fever vaccination is complex and includes :
  - Multiple components of the innate immune system i.e Complement, Interferons , TLRs
  - Multiple cells of the innate immune system i.e NK cells , macrophages , DCs
  - An early (day 3 and day 7) persistent mixed TH1 and TH2 responses
  - Neutralizing antibodies

The Yellow fever vaccine response

- None of these responses could on its own predict protection
  - Individuals with broad and restricted CD4 and CD8 responses are protected
  - Individuals with high and low neutralizing antibody titers are protected
  - Induction of the inflammasome can predict control of viremia
- **The whole is greater than the sum of the parts**

Common Genes expressed in YF and HepA

Symbol	FC 28vs 0		Name	Comment
	YF	HA		
HBA1	2.43	2.39	hemoglobin, alpha 1 (HBA1)	Hemoglobin alpha
HBB	2.21	1.57	hemoglobin, beta (HBB)	oxigen transport, metal Binding
GREM1	1.89	1.54	gremlin 1, cysteine knot superfamily, homolog (Xenopus laevis) (GREM1)	development, cytokine
FABP4	1.82	1.86	fatty acid binding protein 4, adipocyte (FABP4)	transport, acid binding
EGR2	1.71	1.49	early growth response 2 (Krox-20 homolog, Drosophila) (EGR2)	Early growth response
LPL	1.64	1.65	lipoprotein lipase (LPL)	signaling
LMNA	1.56	1.33	lamin A/C (LMNA), transcript variant 2	protein binding
CA2	1.56	1.35	carbonic anhydrase II (CA2)	calcuim dependent activator
RSAD2	1.53	1.51	radical S-adenosyl methionine domain containing 2 (RSAD2)	IFN-response
CLEC12A	1.43	1.51	C-type lectin domain family 12, member A (CLEC12A), transcript variant 3	Important for DC presentation
ATF3	1.43	1.74	activating transcription factor 3 (ATF3), transcript variant 2	Activator transcrition factor (down stream of MAP and EGR)
CD36	1.38	1.48	CD36 antigen (collagen type I receptor, thrombospondin receptor) (CD36), transcript variant 1	innate immune response
SGK	1.37	1.59	serum/glucocorticoid regulated kinase (SGK)	Akt pathway/phophorylate FOXO3a
MPP1	1.36	1.53	membrane protein, palmitoylated 1, 55kDa (MPP1)	signaling/induce NFKB stimulation
TNFRSF12A	1.34	1.34	tumor necrosis factor receptor superfamily, member 12A (TNFRSF12A)	signal thru nfkb and activate bcl2 and bcl-xl
PLEC1	1.33	1.36	plectin 1, intermediate filament binding protein 500kDa (PLEC1), transcript variant 2	lymphocyte extravasation
HSPA1B	1.33	1.33	heat shock 70kDa protein 1B (HSPA1B)	anti-apoptotic
HBG2	1.32	5.46	hemoglobin, gamma G (HBG2)	oxygen transport, metal binding
HBG1	1.31	5.23	hemoglobin, gamma A (HBG1)	oxygen transport, metal binding

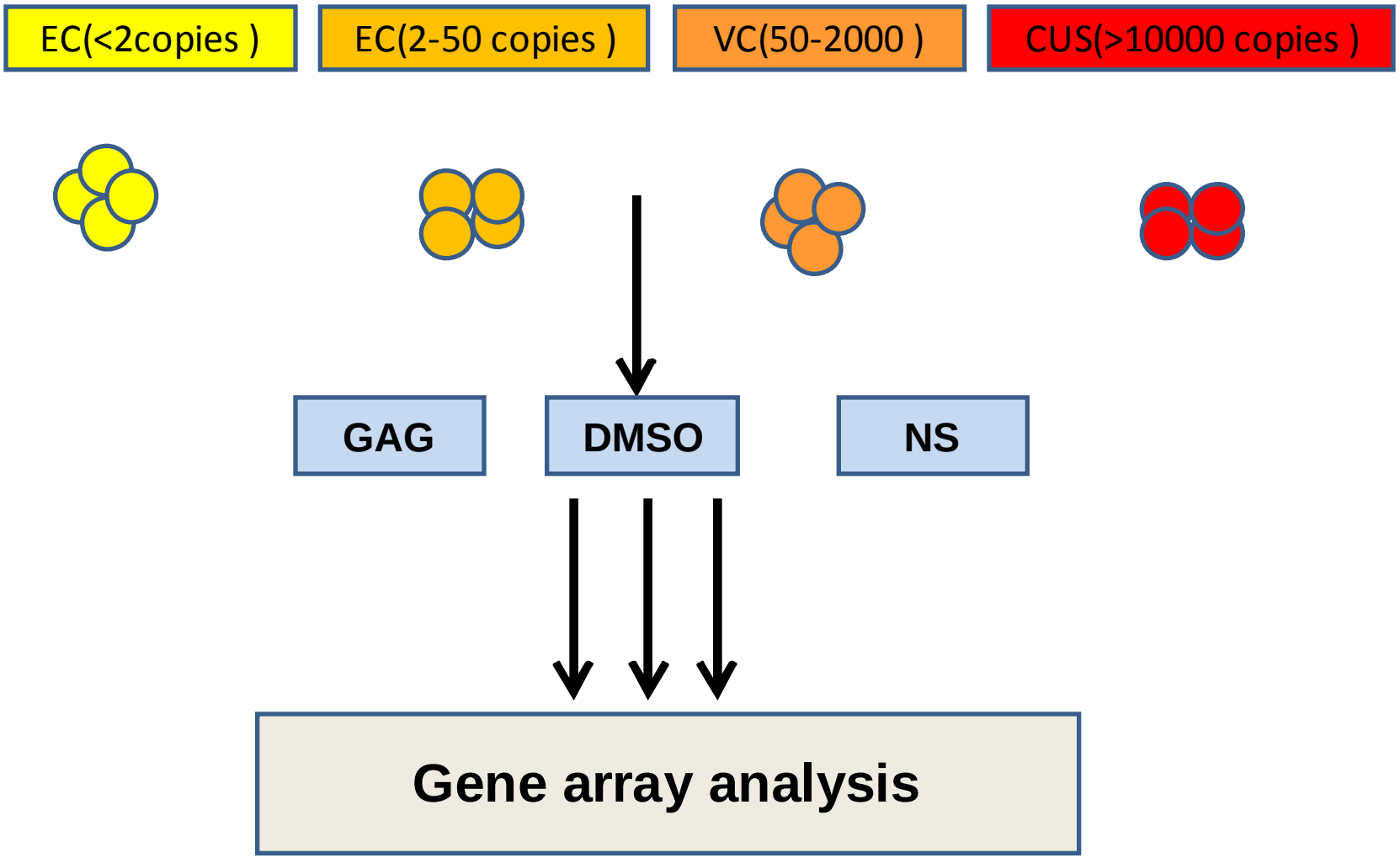
Genes expressed in Elite Controllers

MAP3K5	mitogen activated protein kinase	0.00	pos
IKZF1	IKAROS family zinc finger 1 (Ikaros)	0.00045	pos
CCR5	chemokine (C	0.0043	pos
PSMB7	proteasome (prosome, macropain) subunit	0.0043	pos
LY9	lymphocyte antigen 9	0.0043	pos
PDPK1	PDPK1-3-phosphoinositide dependent protein ki	0.0043	pos
WSB2	WD repeat and SOCS box containing protei	0.006	pos
PTPN22	protein tyrosine phosphatase, non	0.0065	pos
RASGRP1	RAS guanyl releasing protein 1 (calci	0.007	pos
LTBR	lymphotoxin beta receptor (TNFR superfam	0.009	pos
RGS14	regulator of G	0.01	pos
DAB2	disabled homolog 2, mitogen	0.01	pos
CD2BP2	CD2 antigen (cytoplasmic tail) binding	0.01	pos
MYCBP2	MYC binding protein 2	0.02	pos
SRPK1	SFRS protein kinase 1	0.02	pos
DOK1	docking protein 1, 62kDa (downstream of	0.02	pos
GZMK	granzyme K (serine protease, granzyme 3;	0.02	pos
GZMA	granzyme A (granzyme 1, cytotoxic T	0.02	pos
DLG1	discs, large homolog 1 (Drosophila)	0.029	pos
PPP2R1A	protein phosphatase 2 (formerly 2A),	0.028	pos
IL15RA		0.027	pos
YWHAZ	monooxygenase/tryptophan 5	0.034	pos
NOTCH2	Notch homolog 2 (Drosophila)	0.036	pos
MARK3	MAP/microtubule affinity	0.038	pos
TGIF	TGFB	0.038	pos
CAMK2G	calcium/calmodulin	0.03	pos

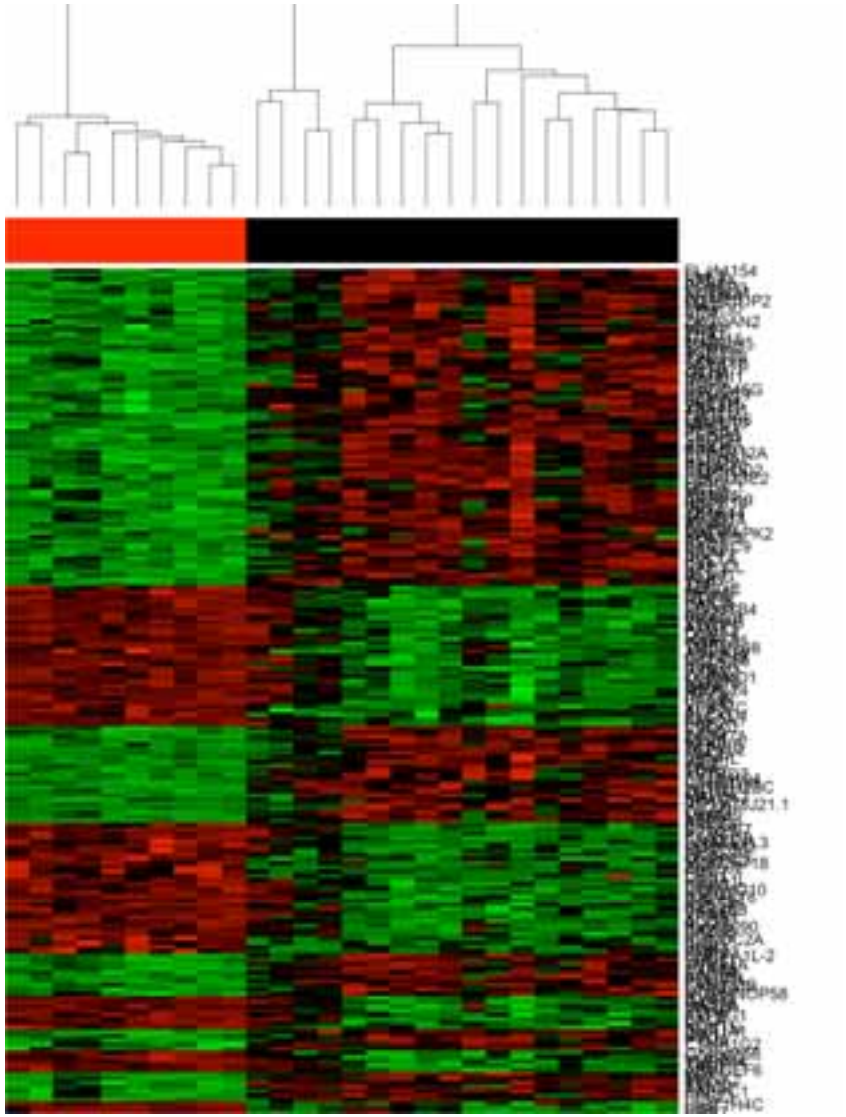
Identification of Gene expression signatures of Immune mediated correlates of protection

The Elite controller study

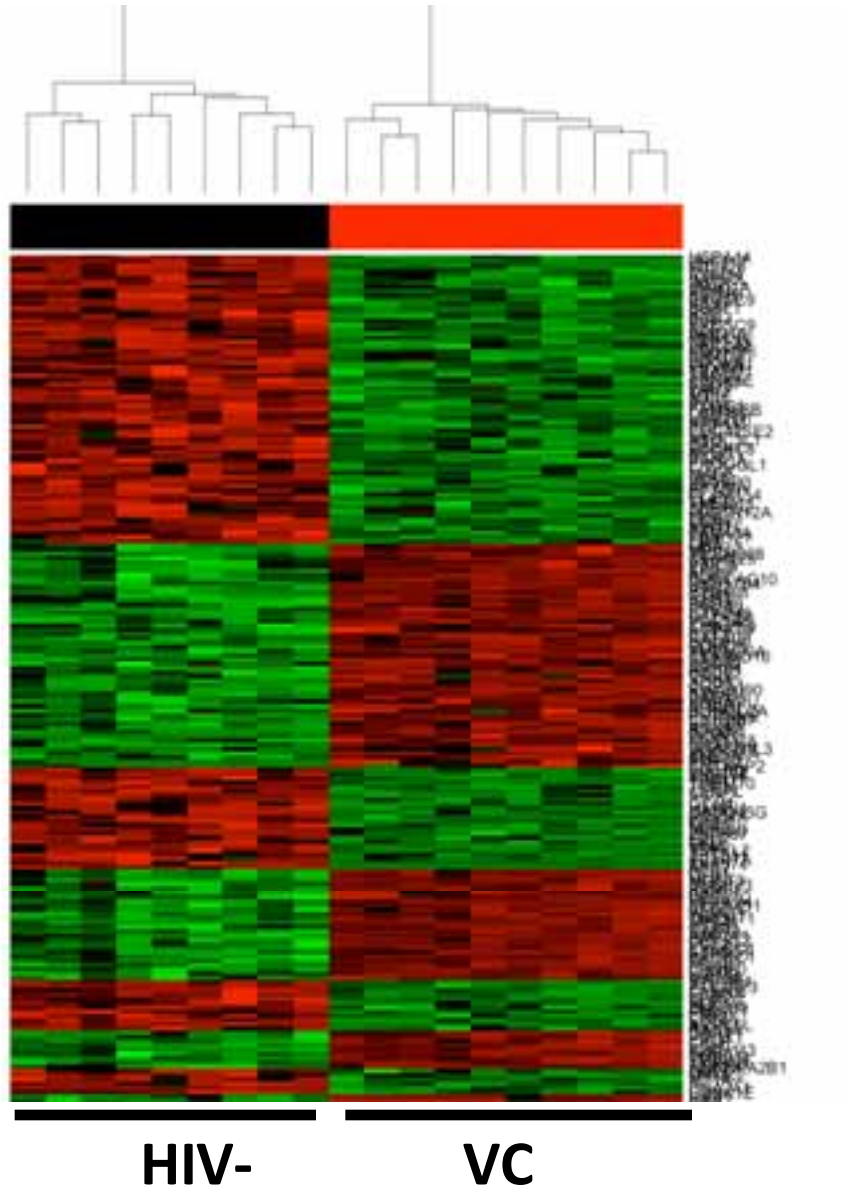
Determine the unique signature of protective immune response to HIV infection



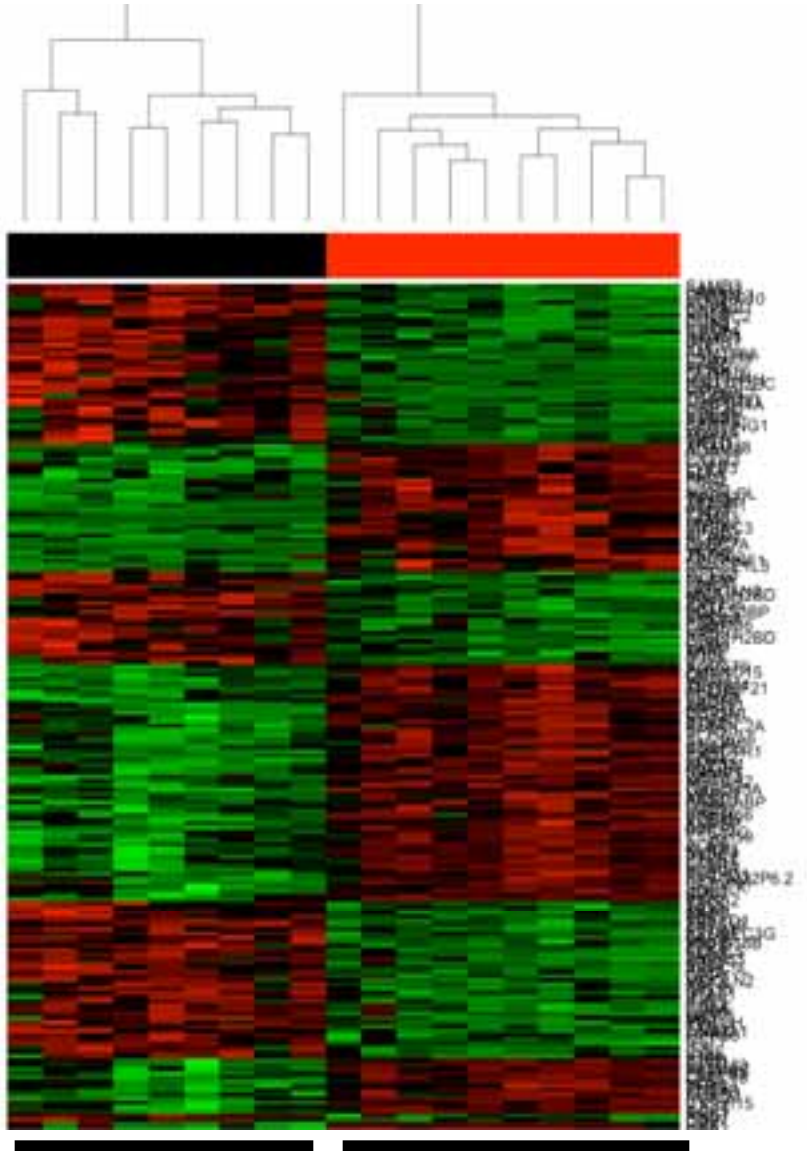
Heat map cluster analysis of EC and HIV- subjects



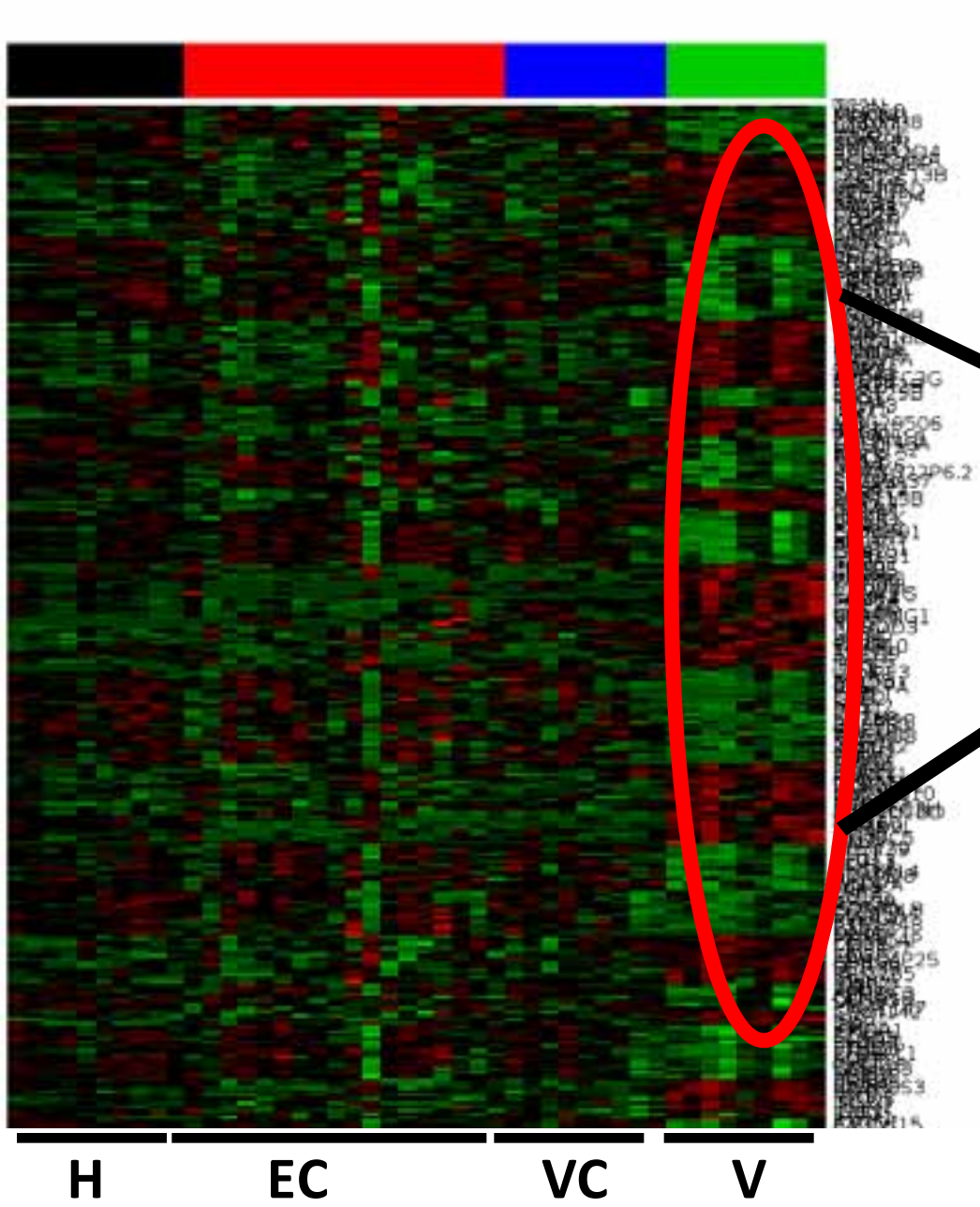
Heat map cluster analysis of VC and HIV- subjects



Heat map cluster analysis of CUS and HIV- subjects

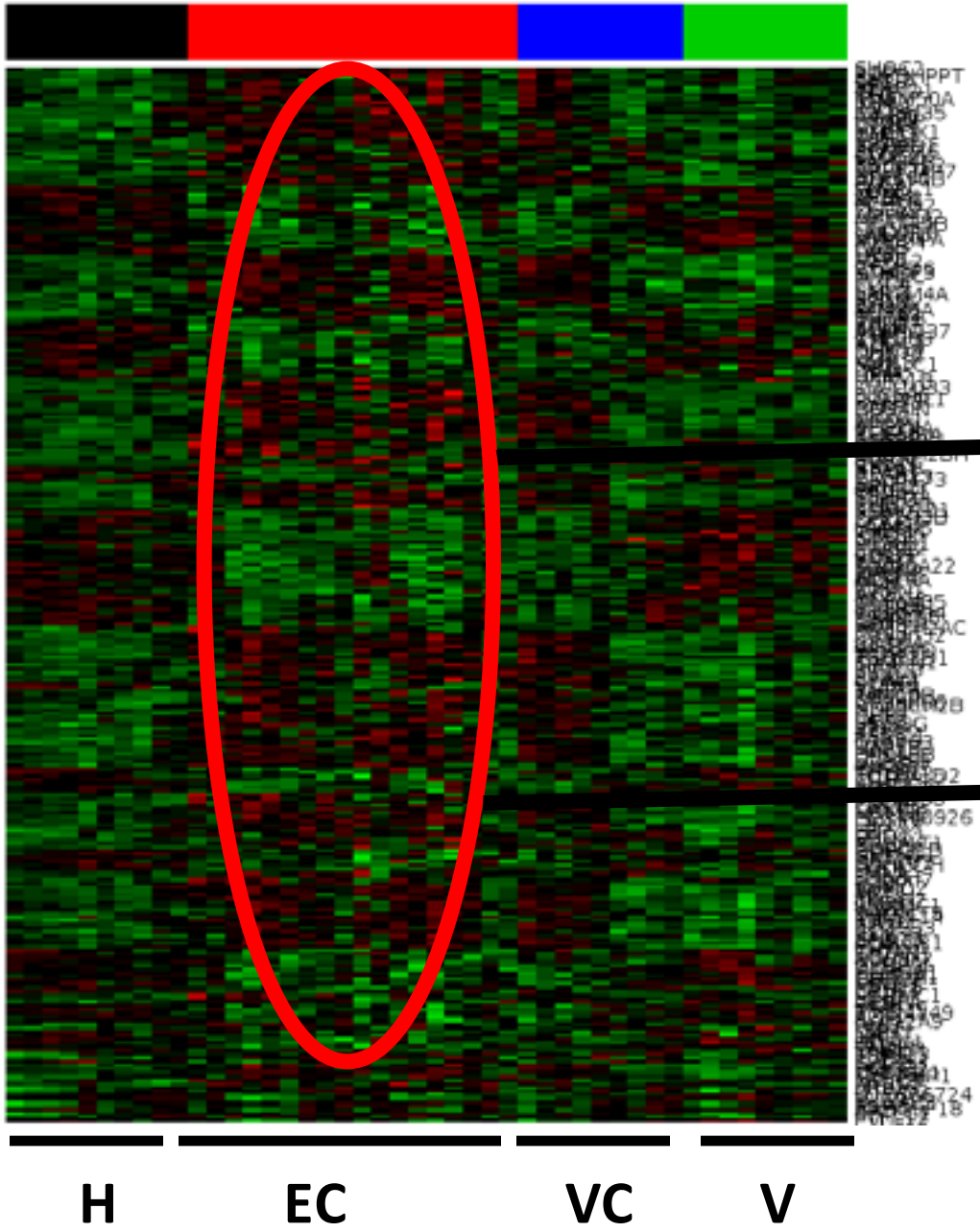


Unique Gene signature of HIV normal viremic progressors



- Interferon induced genes
- Negative receptors (LAIR2, CD160)
- Pro-cycling genes

Unique Gene signature of HIV elite controllers



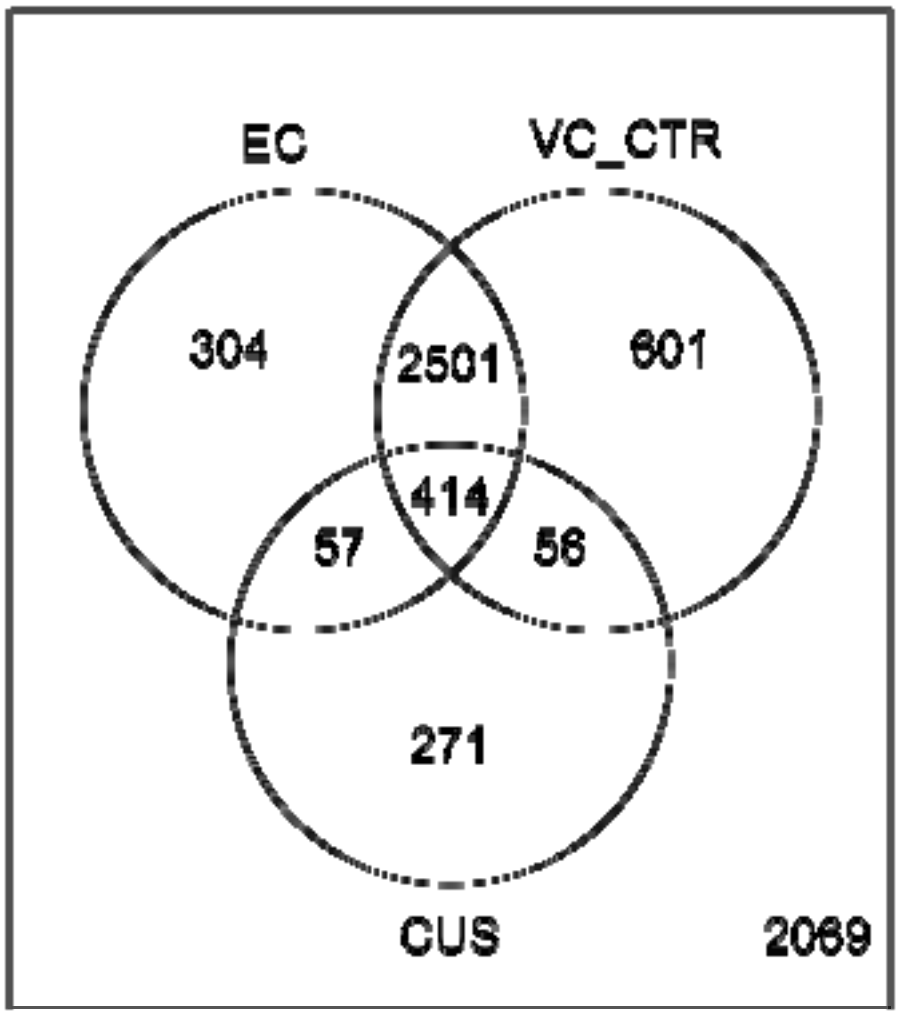
- Survival
- BNIP3L
- Casp6
- Bax
- DC maturation

CLIC4
CLEC2D
CD164

TXN regulation

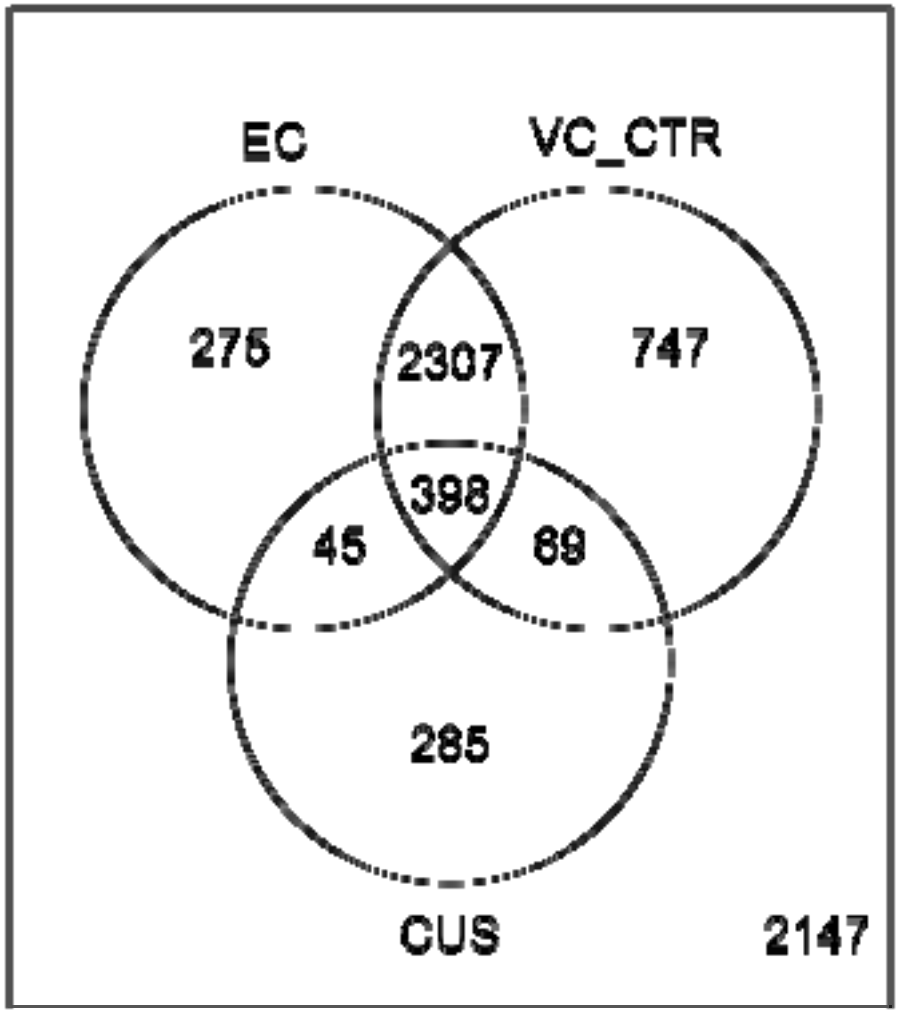
KOX3
SOX4
SOX8

Non stimulated



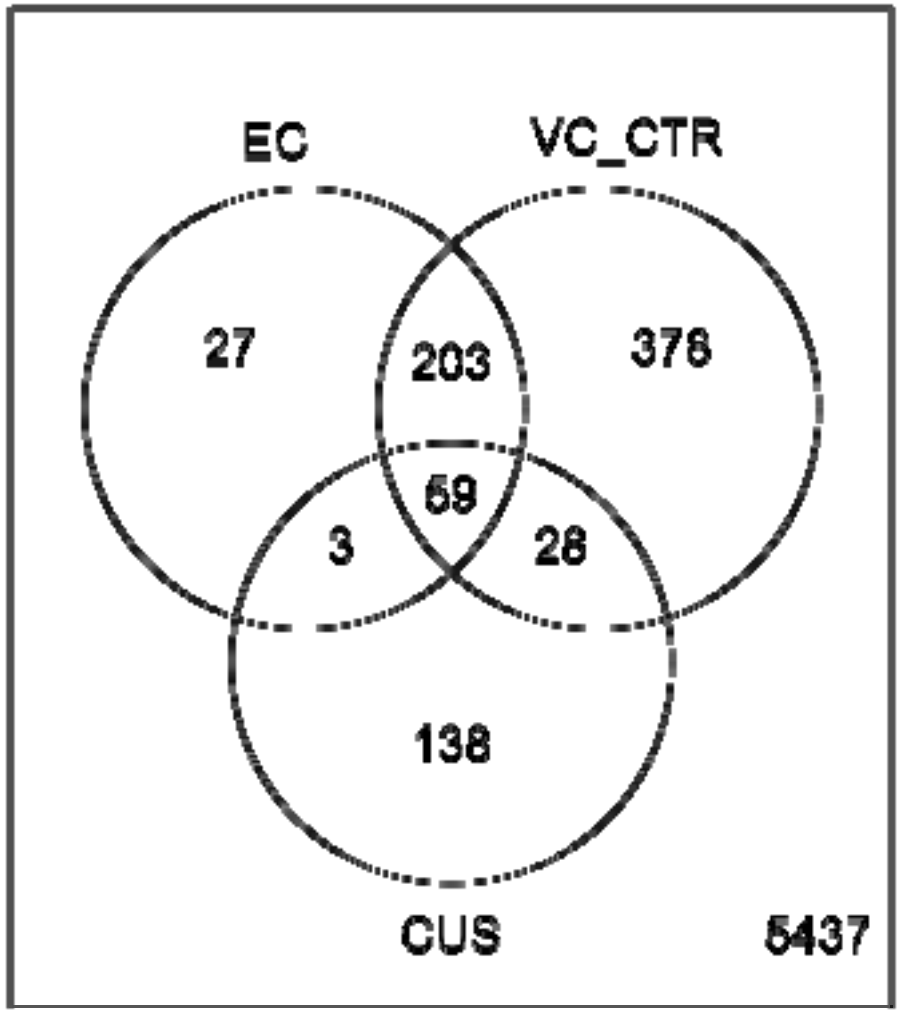
- FDR < 0.05

Non stimulated



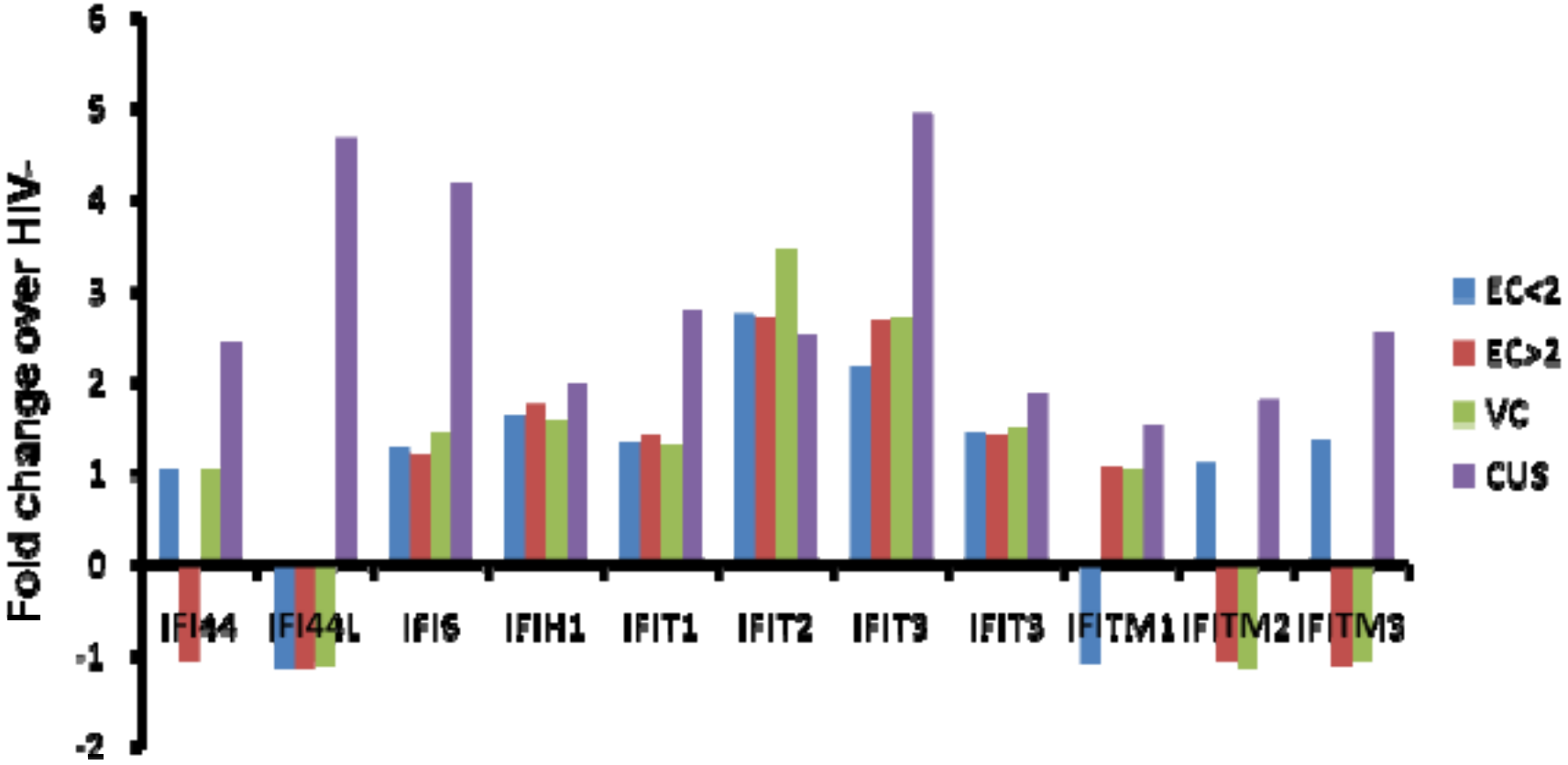
- FDR < 0.05
- |FC | > 1.3

Non stimulated

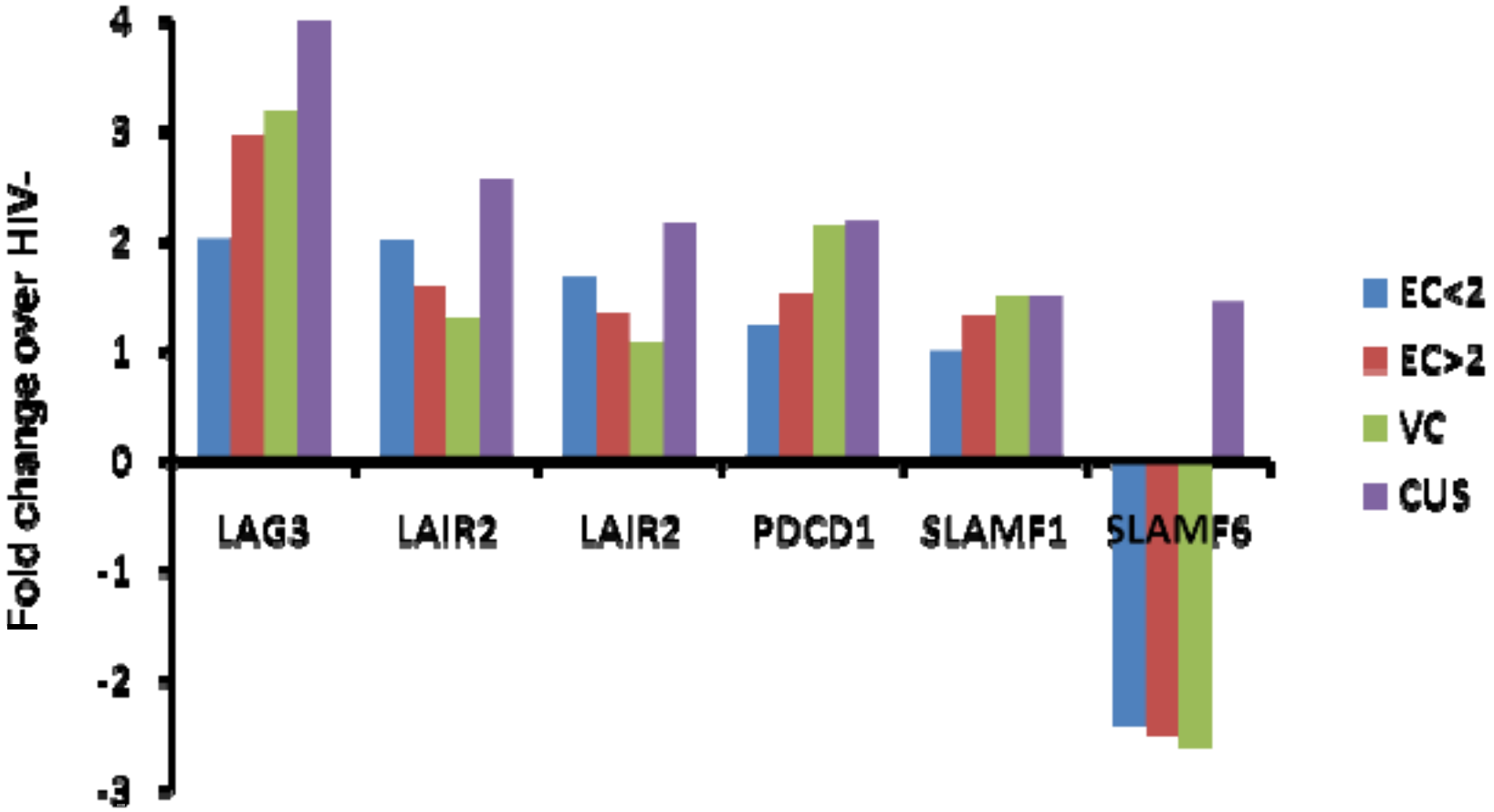


- FDR < 0.05
- |FC | > 2

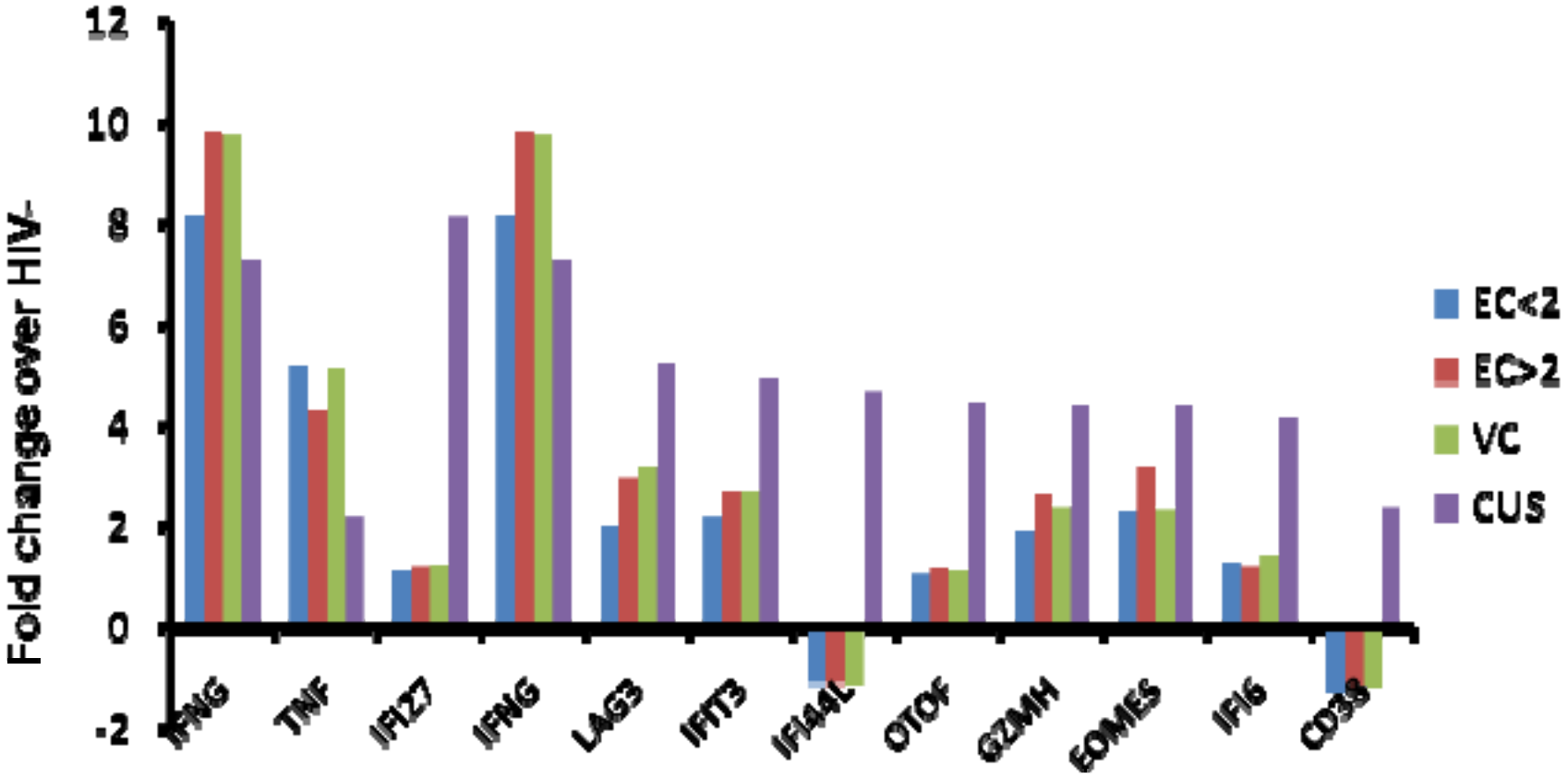
Increased expression levels in Interferon-Induced genes in highly viremic subjects



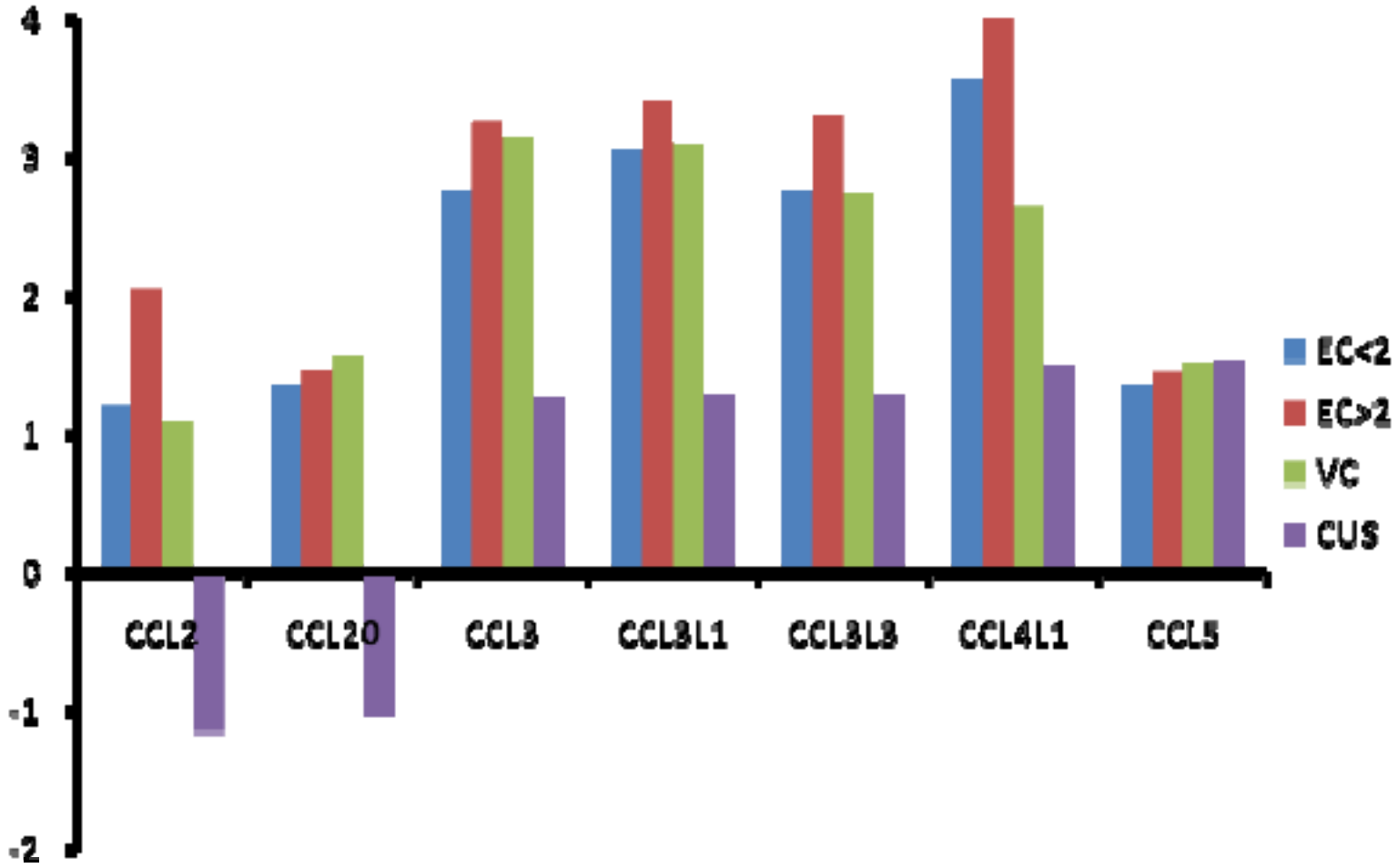
Genes that negatively regulate T cell responses are higher in viremic subjects



Highly viremic subjects express lower levels of cytokine genes



Viremic non-controllers do not express chemokine genes



GSEA Analysis on Non Stimulated PBMC showed similar pathways between viral controllers and Elite controllers

- Common pathways (EC and VC)
  - HoxA5 target genes (T cell function, cell cycling)
  - Survival (DNA repair-related genes and survival factors)
  - TGF-B signaling pathway
  - P38/MAPK signaling pathway
  - P53 signaling pathway
  - ERBB signaling pathway
- Unique CUS pathway
  - IFN-a, IFN-b, IFN-g
  - Cell cycle
  - TCR receptor signaling

Summary

- Identification of HIV specific signature in whole PBMC
- Elite controllers with < 2 copies showed similar transcriptional profile with those between 2 and 50 copies per ml
- Viral controllers share large number of common genes with elite controllers
- Viral controllers share Gene Set Enrichment pathways with elite controllers
- Identification of novel negative regulators (LAIR2, LAIR1, and LAIR3 and CD160).
  - Preliminary results confirms that CD160 triggering inhibits cytokine production
  - Blocking CD160 ligation increases proliferation and cytokine production in CD8+ HIV specific T cells

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