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SAPHIR
Strengthening Animal Production and Health through the Immune Response

UNIVERSITÄT BERN

Assessing vaccine-induced correlates of protection: Antigen-specific readouts versus system vaccinology

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VETERINARY VACCINOLOGY NETWORK

European Veterinary Vaccinology Workshop,
May 9-10, 2016, Ghent, Belgium

PARAGONE

Overview of this workshop

1. Classical readouts of adaptive immune responses
   - Antibodies as correlates of protection (COP)
   - T cells as COP
2. System vaccinology

Correlates of protection: general definitions and considerations

1. Correlates of protection (CoPs): markers which statistically correlates with vaccine efficacy. Are used to predict the protective value of a vaccine.
2. Mechanistic CoP (mCoP): CoP mechanistically and causally responsible for protection
3. Nonmechanistic CoP (nCoP): CoP not involved in the protective effects induced by the vaccine
4. CoPs are valid only for a defined vaccine, species, age group...
5. Understanding pathogenesis is required to define CoP‘s

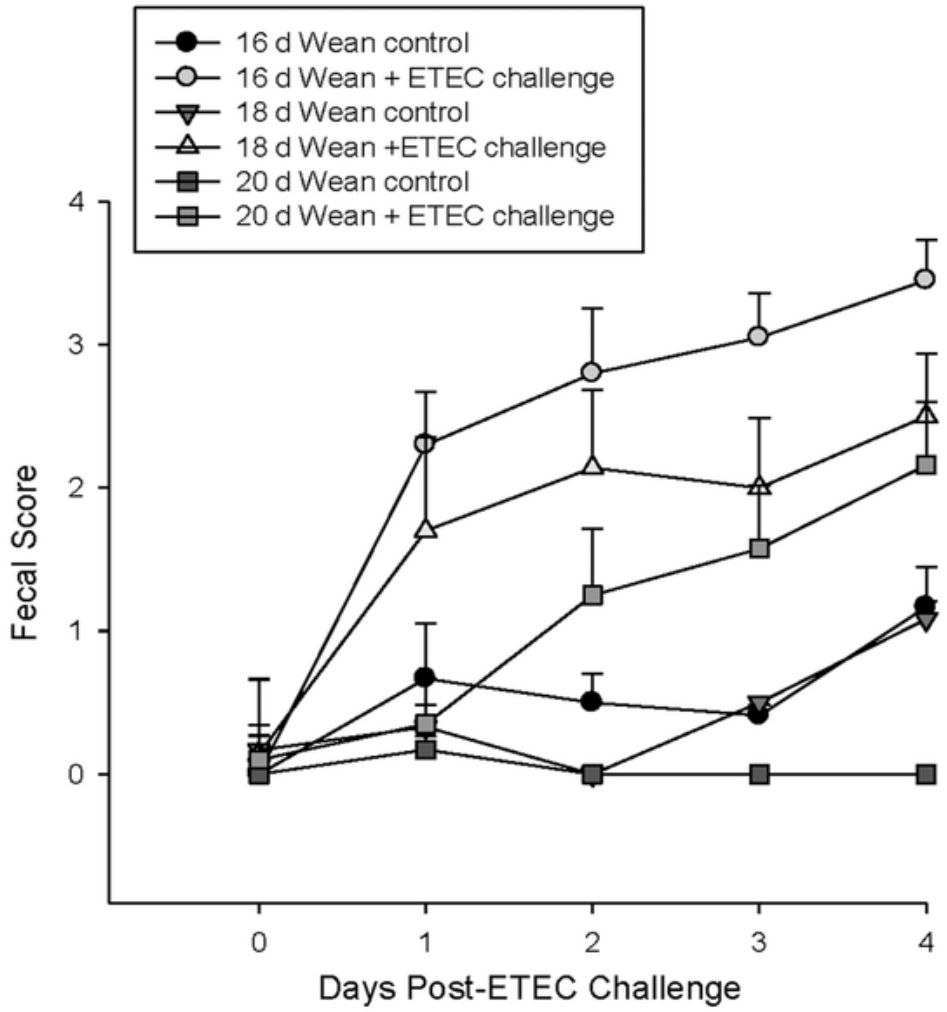
Adapted from Plotkin & Gilbert, Clin. Inf. Dis. 2012 Jun;54(11):1615-7

Human CoP

Vaccine	Test	Level required
Anthrax	Toxin neutralization	1,000 IU/ml
Diphtheria	Toxin neutralization	0.01–0.1 IU/ml
Hepatitis A	ELISA	10 mIU/ml
Hepatitis B	ELISA	10 mIU/ml
Hib polysaccharides	ELISA	1 μg/ml
Hib conjugate	ELISA	0.15 μg/ml
Human papillomavirus	ELISA	ND[b]
Influenza	HAI	1/40 dilution
Japanese encephalitis	Neutralization	1/10 dilution
Lyme disease	ELISA	1,100 EIA U/ml
Measles	Microneutralization	120 mIU/ml
Meningococcal	Bactericidal	1/4 (human complement)
Mumps	Neutralization?	ND
Pertussis	ELISA (toxin)	5 units
Pneumococcus	ELISA; opsonophagocytosis	0.20–0.35 μg/ml (for children); 1/8 dilution
Polio	Neutralization	1/4–1/8 dilution
Rabies	Neutralization	0.5 IU/ml
Rotavirus	Serum IgA	ND
Rubella	Immunoprecipitation	10–15 mIU/ml
Tetanus	Toxin neutralization	0.1 IU/ml
Smallpox	Neutralization	1/20
Tick-borne encephalitis	ELISA	125 IU/ml
Tuberculosis	Interferon	ND
Varicella	FAMA gp ELISA	≥1/64 dilution; ≥5 IU/ml
Yellow fever	Neutralization	1/5
Zoster	$CD4^+$ cell; lymphoproliferation	ND

[a] Also see the text.
[b] ND, not defined.

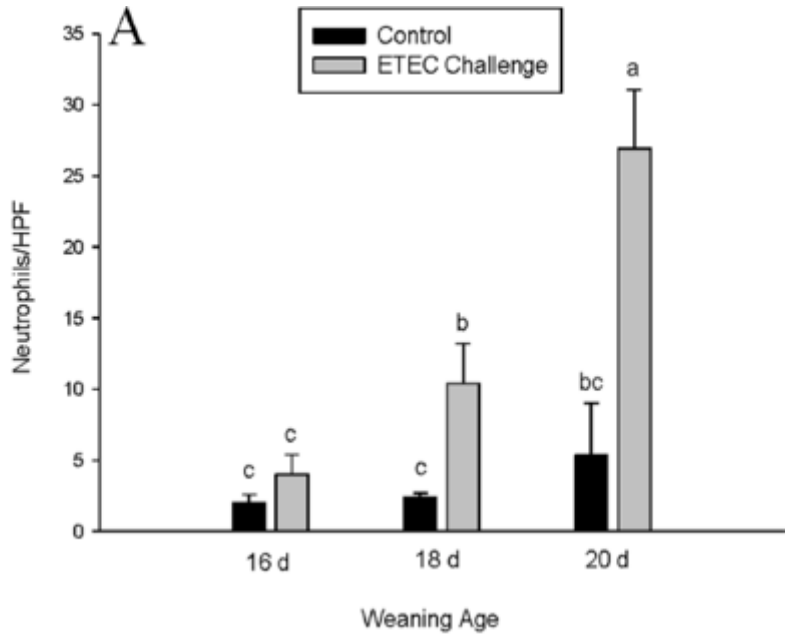
Immunopathogenesis versus protective immune responses

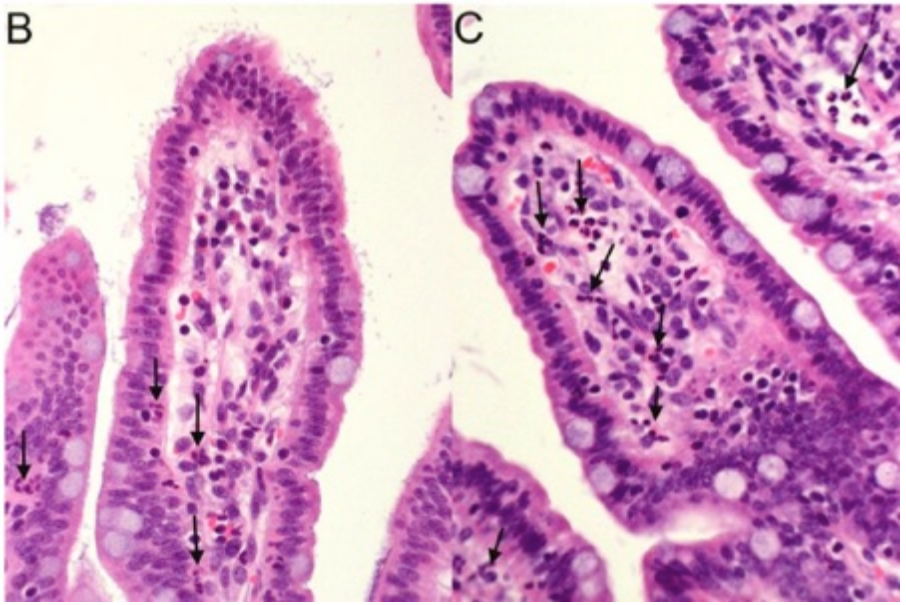


Question: What is the relationship of fecal scores and the inflammatory status in the gut?

McLamb et al., 2013 PlosOne 8(4): e59838. doi:10.1371/journal.pone.0059838

Understanding the protective immune response



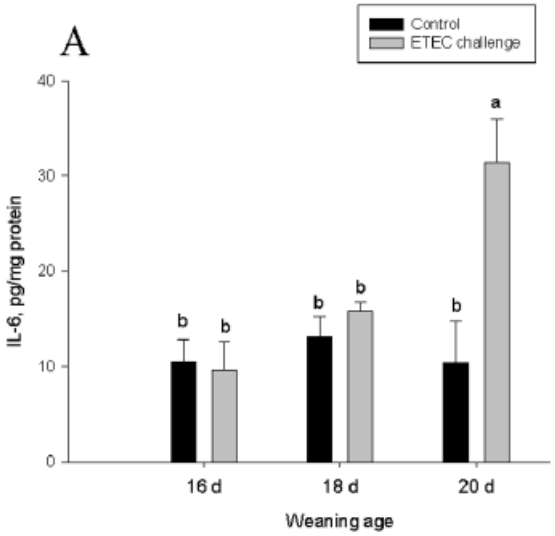


Increased neutrophil recruitment associated with enhanced resistance to infection of piglets by ETEC

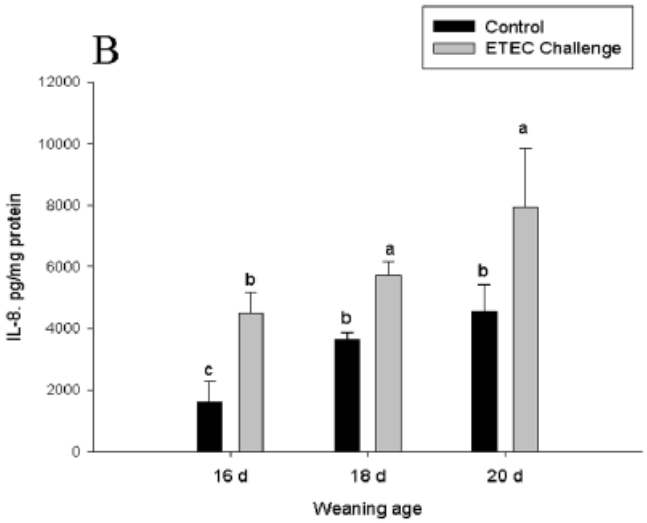
McLamb et al., 2013 PlosOne 8(4): e59838. doi:10.1371/journal.pone.0059838

Understanding the protective immune response

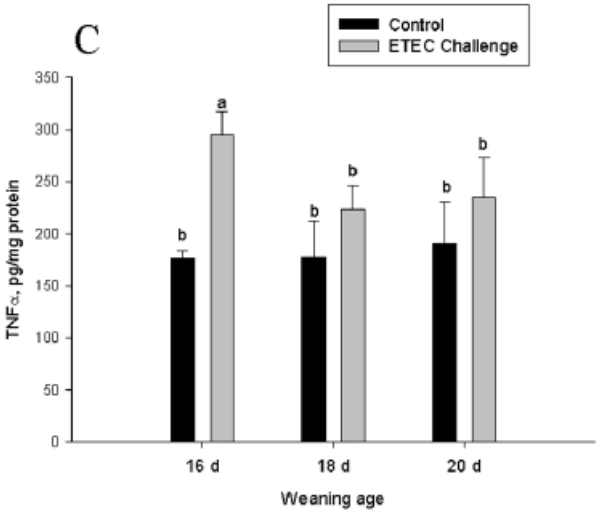
IL-6



IL-8



TNF



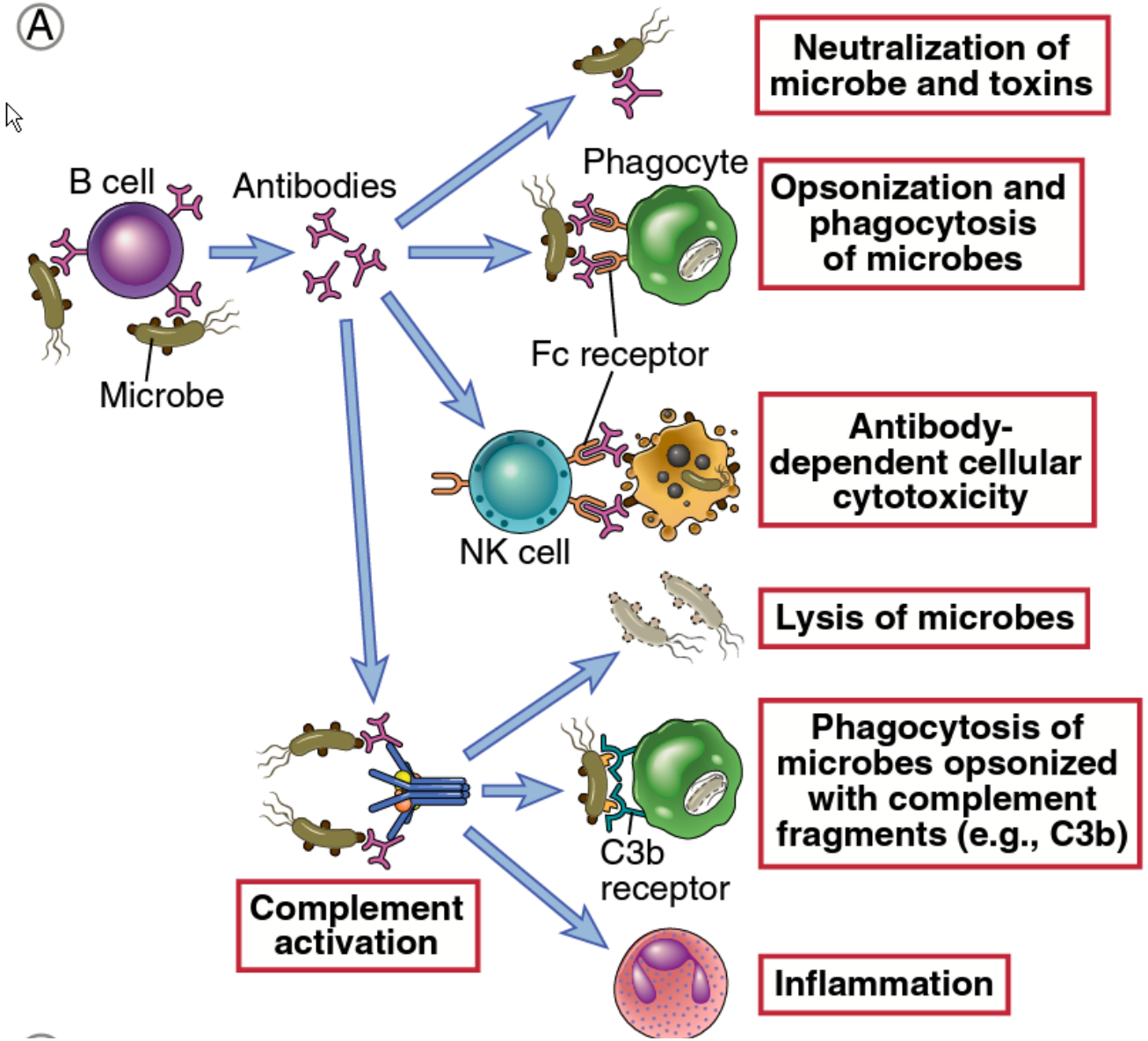
Increased IL-6 responses are associated with enhanced resistance to infection

McLamb et al., 2013 PlosOne 8(4): e59838.

Antibody-based CoP

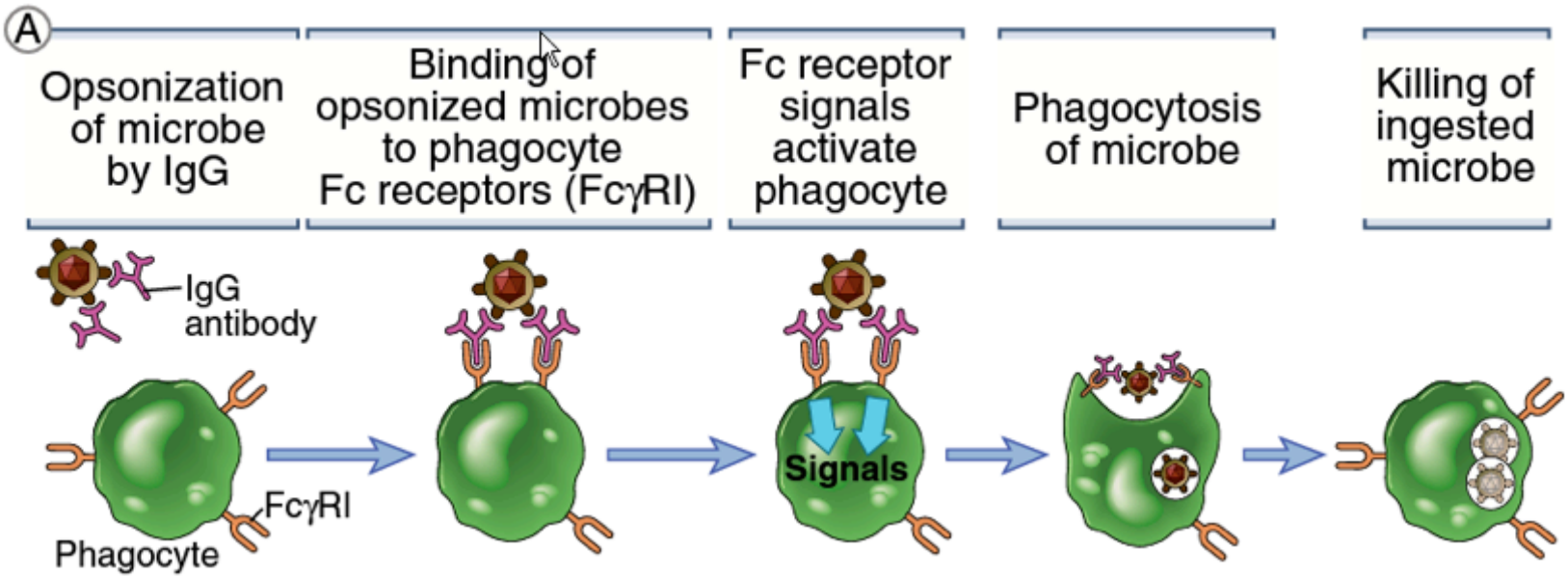
1. ELISA titre: → usually only a nCoP
2. Avidity and affinity: Antigen-antibody interactions are non-covalent based on hydrogen bonds, hydrophobic interactions, electrostatic and van der Waals forces. They are therefore reversible.
   - Affinity: strength of interaction between an epitope and paratope.
   - Avidity: accumulated strength of the antibody-antigen complex, dependent on affinity, valency of both the antibody and antigen and structural arrangement
3. Antibody function → mCoP

Antibody functions



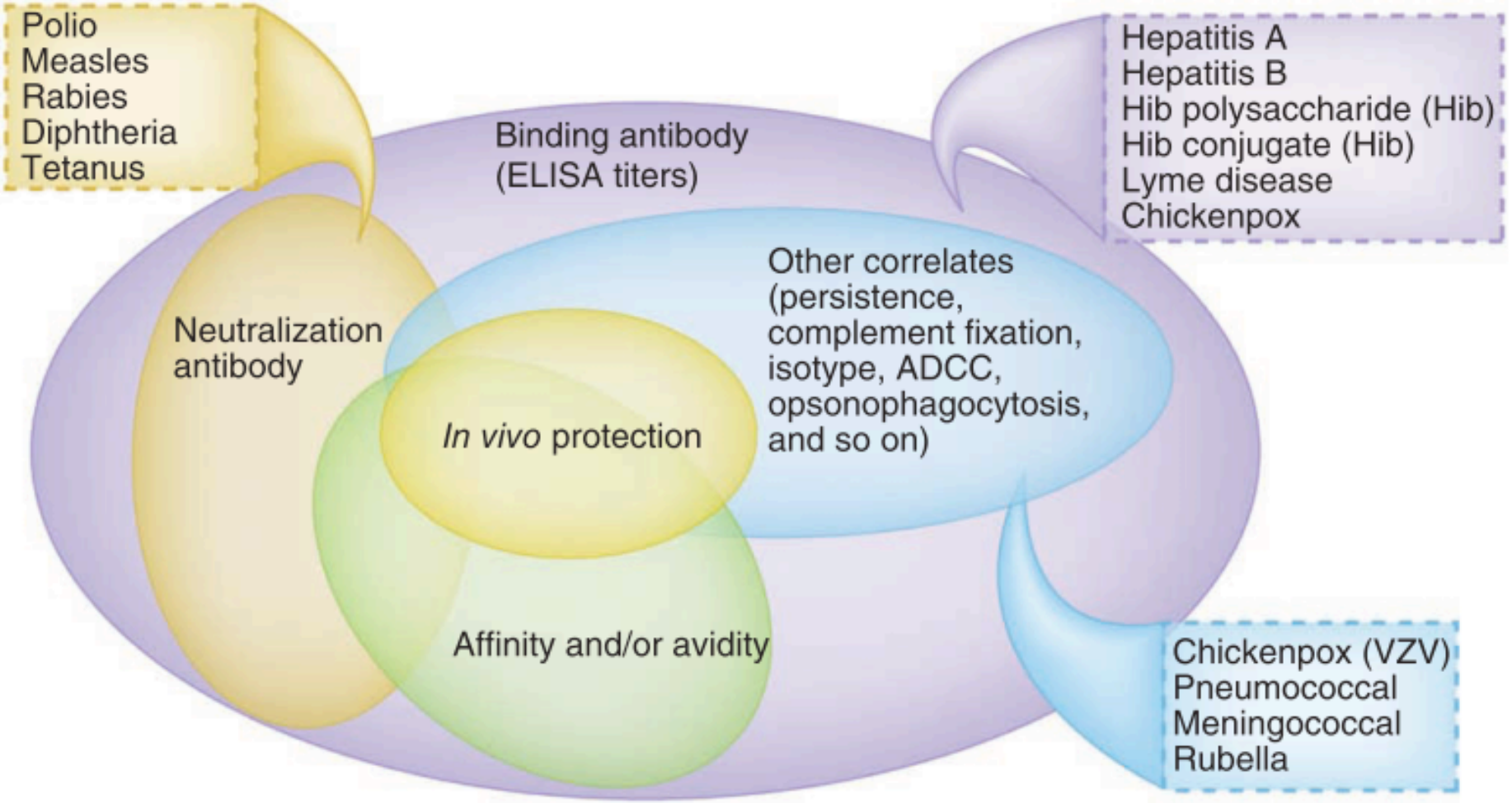
Basic Immunology, Abbas et al, Elsevier Saunders, 9th Edition; Figure 8-1

Antibody-mediated opsonization of microbes



Cytokine release,
Improved antigen presentation

Human antibody-based CoPs



Pulendran & Ahmed, Nat. Immunol. 2011; 12: 509.

What is the relationship between opsonization and neutralization?

"I was able to neutralize the stress hormone using chocolate."

Minimum epitope requirements for functional activity of Mab D9 recognizing linear epitope on VP1 of FMDV

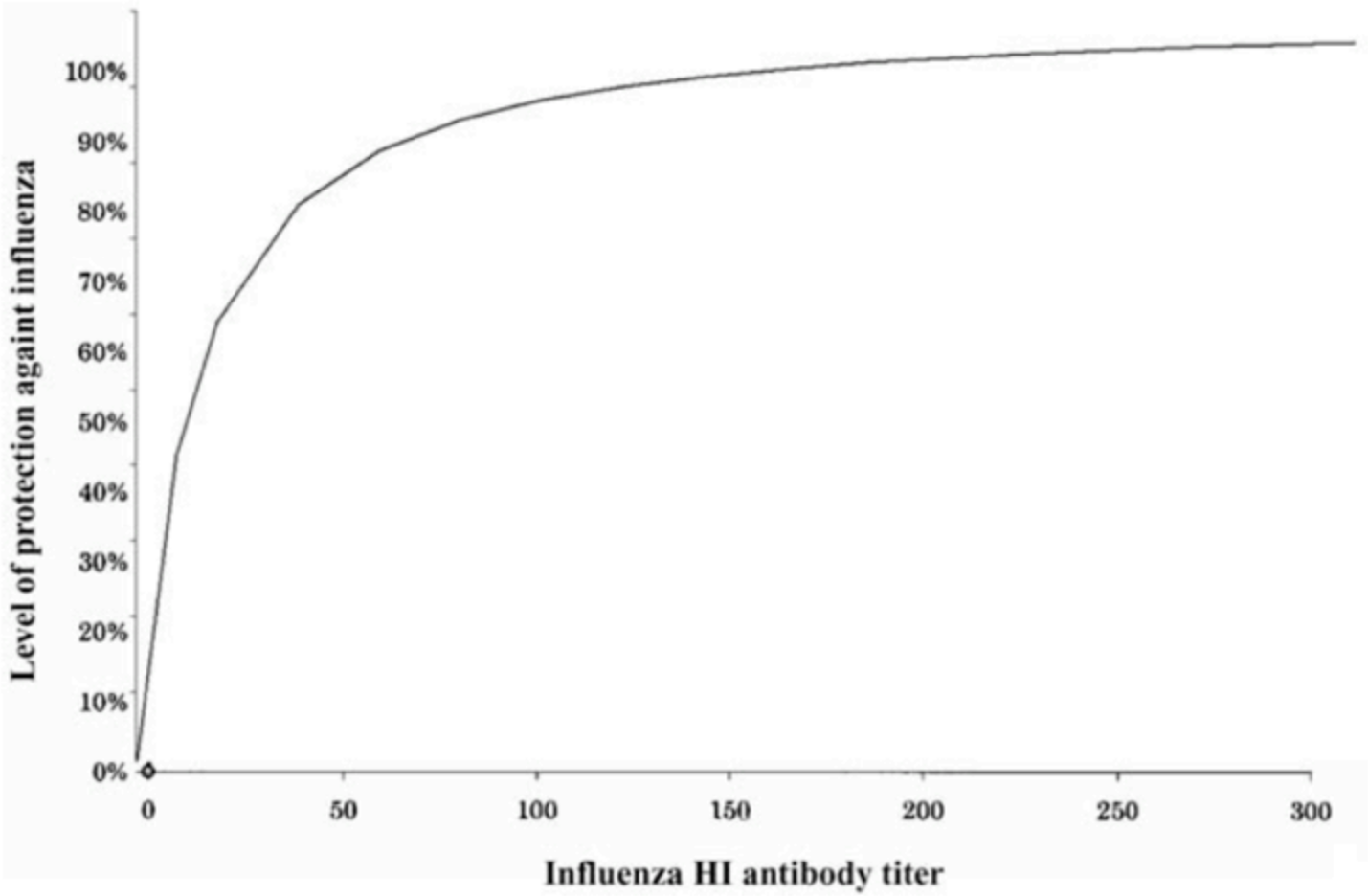
*P*OSITION 144 -154 VP1: NEUTRALIZING EPITOPE WITH RECEPTOR BINDING SITE

		Neut	Ops	ELISA
O1 Kaufbeuren	LRGDLQVLAQKV	+	+++	+
O Bulgaria 1/91	VRGDLQVLARKA	+	+++	+
O Grece 23/94	VRGDLQVLARKA	+	+++	+
O Grece 22/96	VRGDLQVLAQKA	+	+++	+
O Vietnam 7/97	VRGDLQVLAQKA	+	+++	+
A Macedonia 6/96	TRGDLGQLAART	–	+++	–
A 24 Cruzeiro	RRGDMGSLAARV	–	+	–
Asia-1 Turkey/2000	RRGDMAALTQRL	–	+	–
C-S8cl	ARGDLAHLTTTH	–	++	–
Asia 1 Shamir	RRGDMAALAQRL	–	–	–

Antibody-based CoP: what else is important

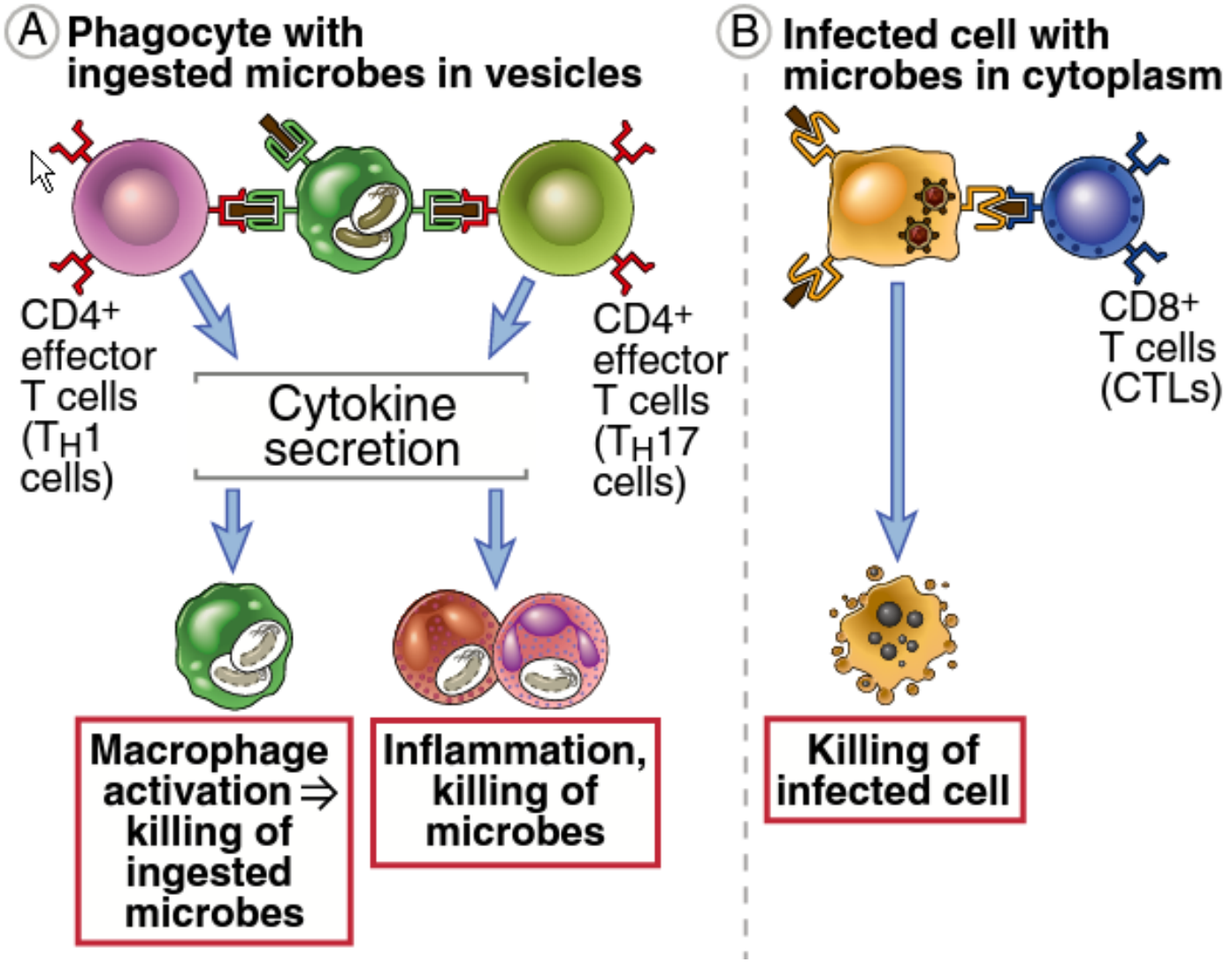
1. Antibody isotype
2. Antibody location: Mucosal antibodies, colostrum/milk antibodies
3. Broadly cross-reactive antibodies: targeting conserved epitopes
4. Mechanisms of neutralization
5. Frequency of memory B cells → ELISPOT
6. Frequency of plasma cells → ELISPOT

Example for influenza



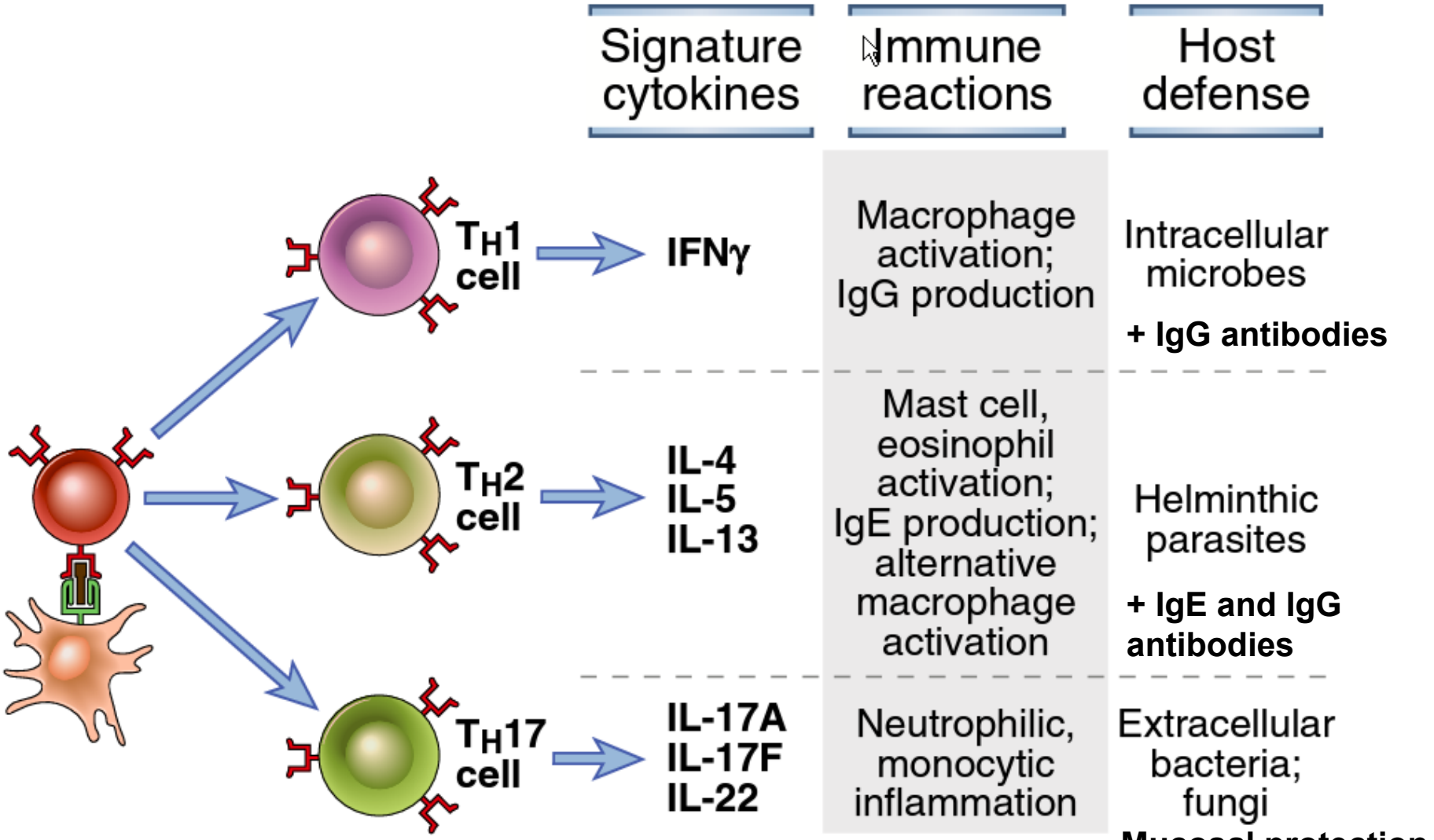
In adults a titre of 1:40 predicts 80% protection
BUT in children 1:330 predicts 80% protection

T-cell-based CoP



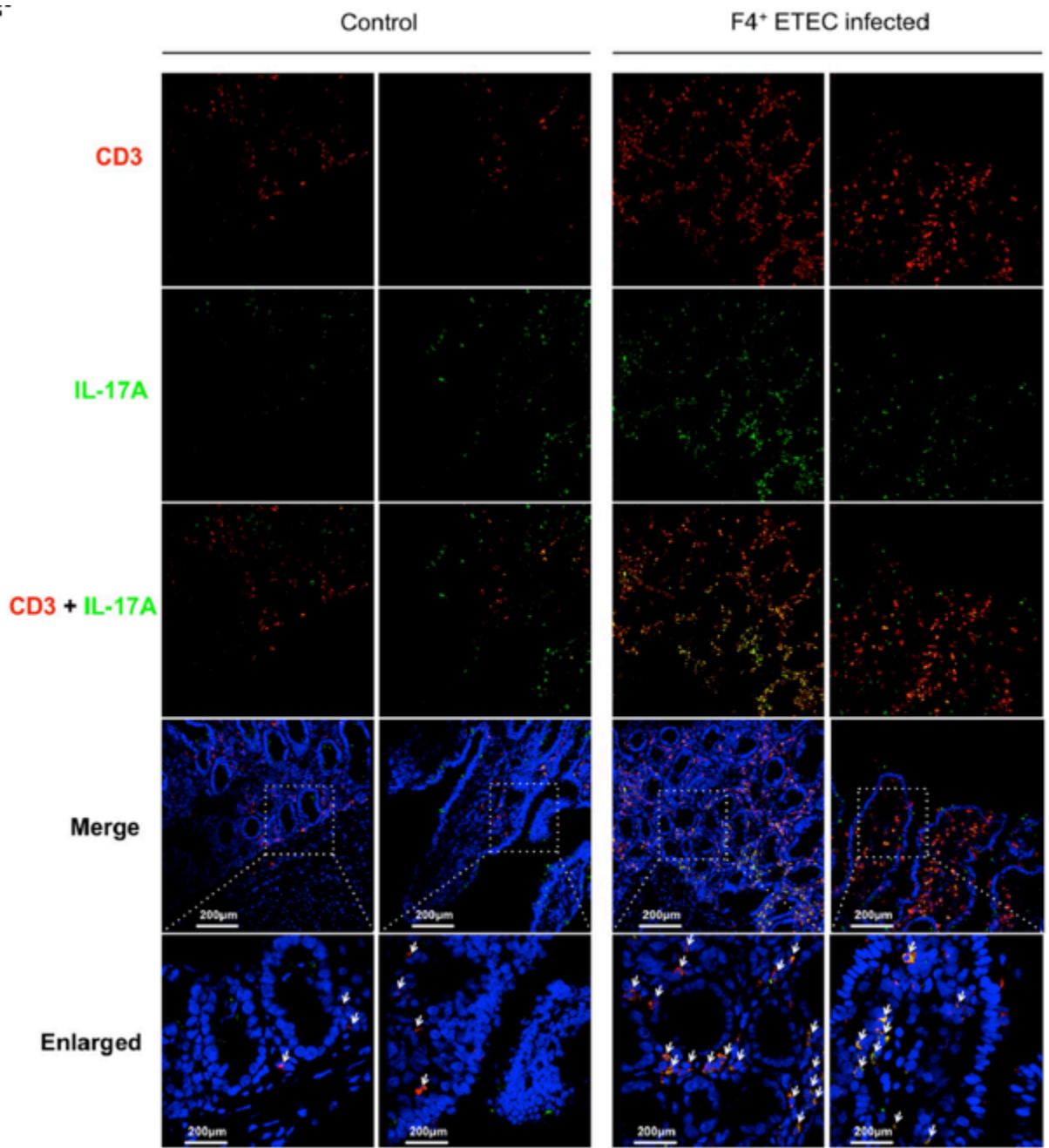
Basic Immunology, Abbas et al, Elsevier Saunders, 9th Edition; Figure 5-1

CD4 + helper T lymphocytes during infections



Basic Immunology, Abbas et al, Elsevier Saunders, 9th Edition; Figure 5-14

Understand the immune response against you pathogen!



IL-17 dominated immune response induced by ETEC in piglets

Luo et al., 2015, Vet. Res 46:121

T-cell-based CoP

1. T-cell readout need in vitro restimulation
2. Frequency of responding cells
3. Phenotype of responding cells:
   - CD4 or CD8,
   - Expression of homing markers (α4β7 integrin for intestinal mucosal homing)
4. T cell function
   - Expression of individual or multiple cytokines
   - Proliferation
   - Killing capacity, perforin expression...
   - Number of epitopes targeted
   - Sequence conservation of epitopes targeted

Proposed immune mechanism in protection by veterinary vaccines

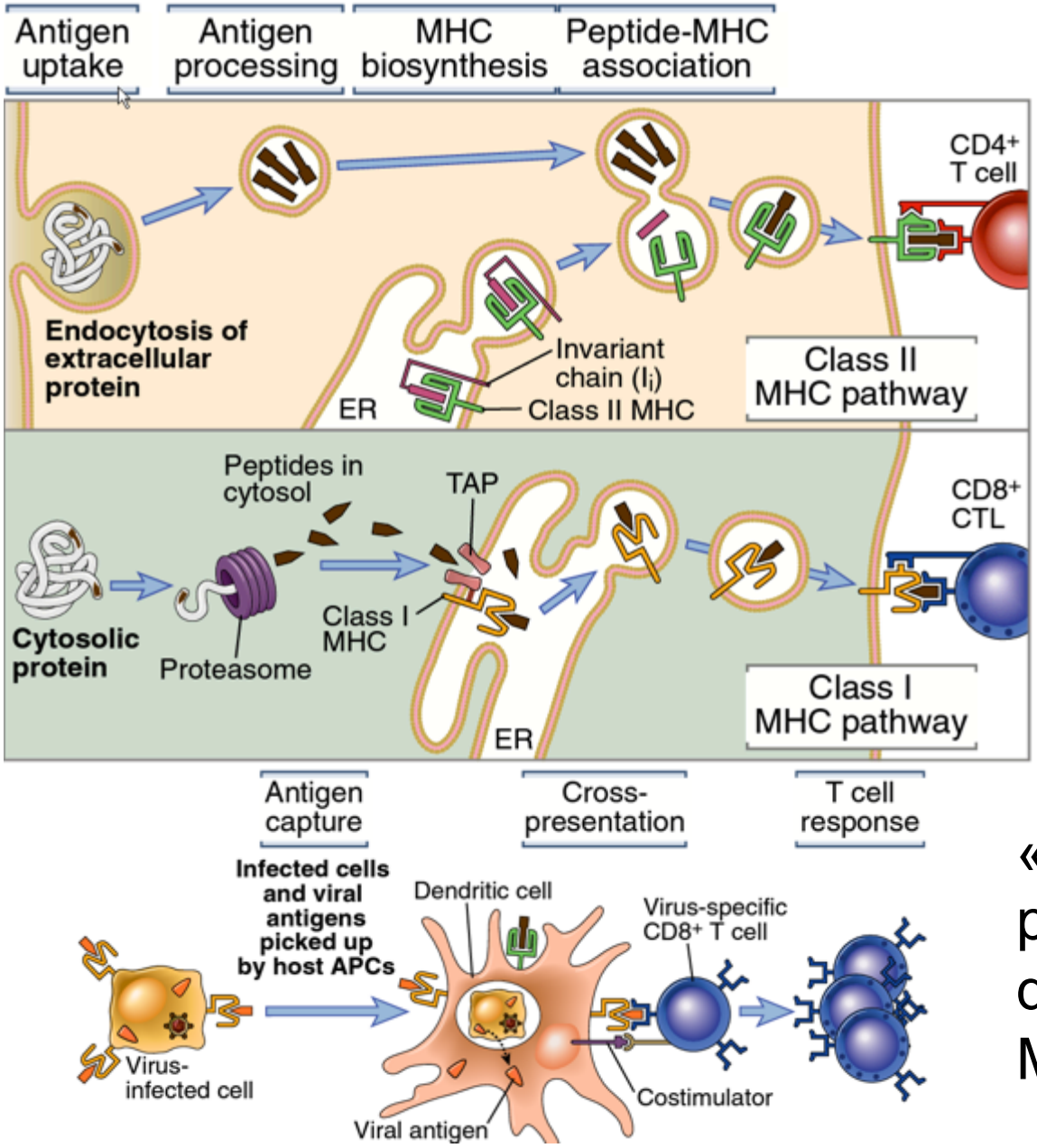
DISEASE	Antibody	T cells
Cytolytic virus infection and extracellular bacterial infections		
FMDV, Bluetongue, Rotavirus, Parvovirus, Rabies, Swine Influenza		
Clostridium, E. Coli...		
Non-cytolytic and persisting viruses and persisting parasites and bacteria		
Neospora		CTL, Th1
Theileria		CTL, Th1
Paratuberculosis, Tuberculosis		Th1
PRRSV, CSFV, BVDV, BHV1, PRV		CTL, IFNγ

essential
important
minor role

Facts on T lymphocytes

1. Specificity:
   - Crossreactivity! One TCR can recognize multiple epitopes through molecular mimicry
2. Activation:
   - in the draining lymph node!
   - 3 divisions per day, total 8-15 to reach peak in 1 week: 10000 fold enrichment
   - Differentiation to effector cells (up to 5% of total T-cell pool!)
3. Memory
   - only 5% of effector cells differentiate in memory cells
   - T cell memory is maintained for long time through homeostatic proliferation (IL-7 and IL-15 mediated)
4. Recirculation: 1-2% of lymphocytes per hour.

Antigen processing and presentation



«Cross-presentation» by dendritic cells for MHC class I

Basic Immunology, Abbas et al, Elsevier Saunders, 9th Edition; Figure 3-12 and 3-16

Use of PBMC to assess T-cell immunity

- ☺ Easy preparation, contain all cells required:
  - → 70% T cells
  - → 10 % B cells
  - → 1% dendritic cells (not many but potent!)
- ☹ Presence of innate cells which may give „background“ (NK cells, γδ T cells): depletion by cell sorting might be required
- ☹ Presence of suppressor cells (monocytes, regulatory T cells
- ☹ Antigen presentation not always possible or effective

Selection of antigens for in vitro restimulation

1. Live pathogens: possible for some viruses, may be not suitable for complex pathogens.
2. Inactivated pathogens to prevent cytopathogenicity. MHC I presentation?
3. Purified recombinant proteins. MHCI presentation?
4. Peptides. Problem MHC haplotype dependency
5. Gene delivery systems: mRNA transfection, plasmid transfection (works only with cell lines), viral vectors

Consider presence of PAMPS which will influence assay (enhancement or suppression, innate responses, “unspecificity”)

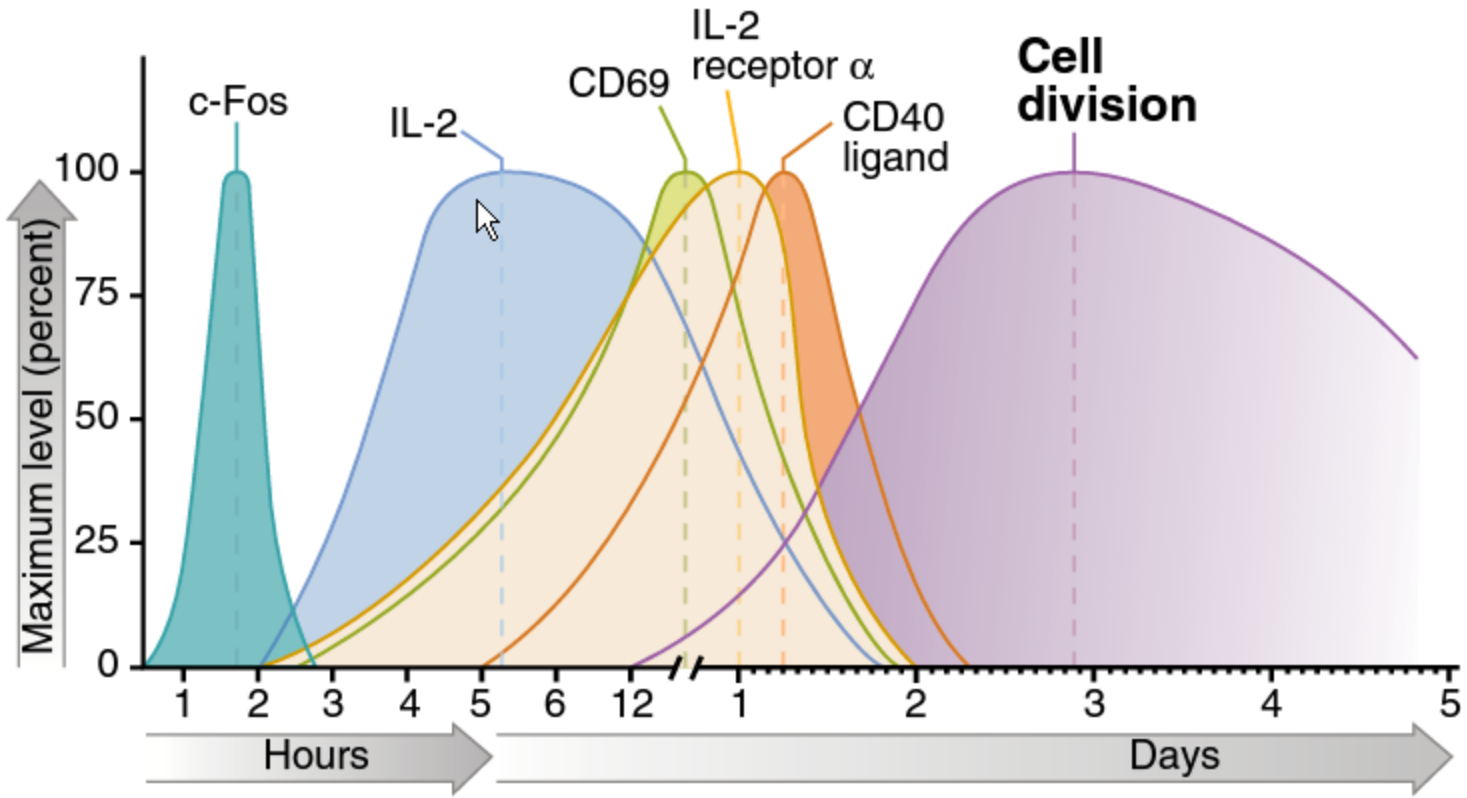
Other important parameters

1. Optimization of medium composition
    1. serum-free medium
    2. Addition of cytokines: IL-2, IL-7
2. Incubation times will depend on antigen system, early or late memory stage
3. Use of frozen cells possible but freezing/thawing procedure needs to be optimized (see Disis et al., 2006 J. Immunol Methods 308). Loss of sensitivity

T-cell assays in veterinary immunology

1. Proliferation assay microplates
   bulk assays: 3H-thymidine incorporation, MTT assay,
   single cell assays: CSFE/Violet Stain, Ki67, BrDU
2. Measurement of activation markers by flow cytometry
3. Cytokine responses:
   - Bulk assay with supernatants: ELISA and Multiplex
   - Bulk assay for frequency: ELISPOT
   - Bulk assay for cytokine mRNA
   - Single cell assay using intracellular cytokine staining by FCM
4. Cytotoxic T-cell assays:
   - Killer assays radioactive (Cr-release assay) or flow cytometry
   - CD107 assay, perforin staining, granzyme B release
- Tetramer staining (MHC haplotype charaterized and tools available)

Proteins produced by antigen-stimulated T cells



Basic Immunology, Abbas et al, Elsevier Saunders, 9th Edition; Figure 5-8

Other considerations for CoP based on T-cell assays

1. Due to constant re-circulation of memory cells, the frequency of antigen specific T cells is highly variable in the blood. Repeated sampling may be necessary as well as enough data for a robust statistical analysis.
2. Memory cells are highly heterogenous (Th1, Th2, Treg, Th17, Tcm, Tem, multiple cytokine producers, mucosal T cells…) and identifying them depends on the methodology selected.
3. Correlates of protection can change with time post vaccination and are age-dependent.

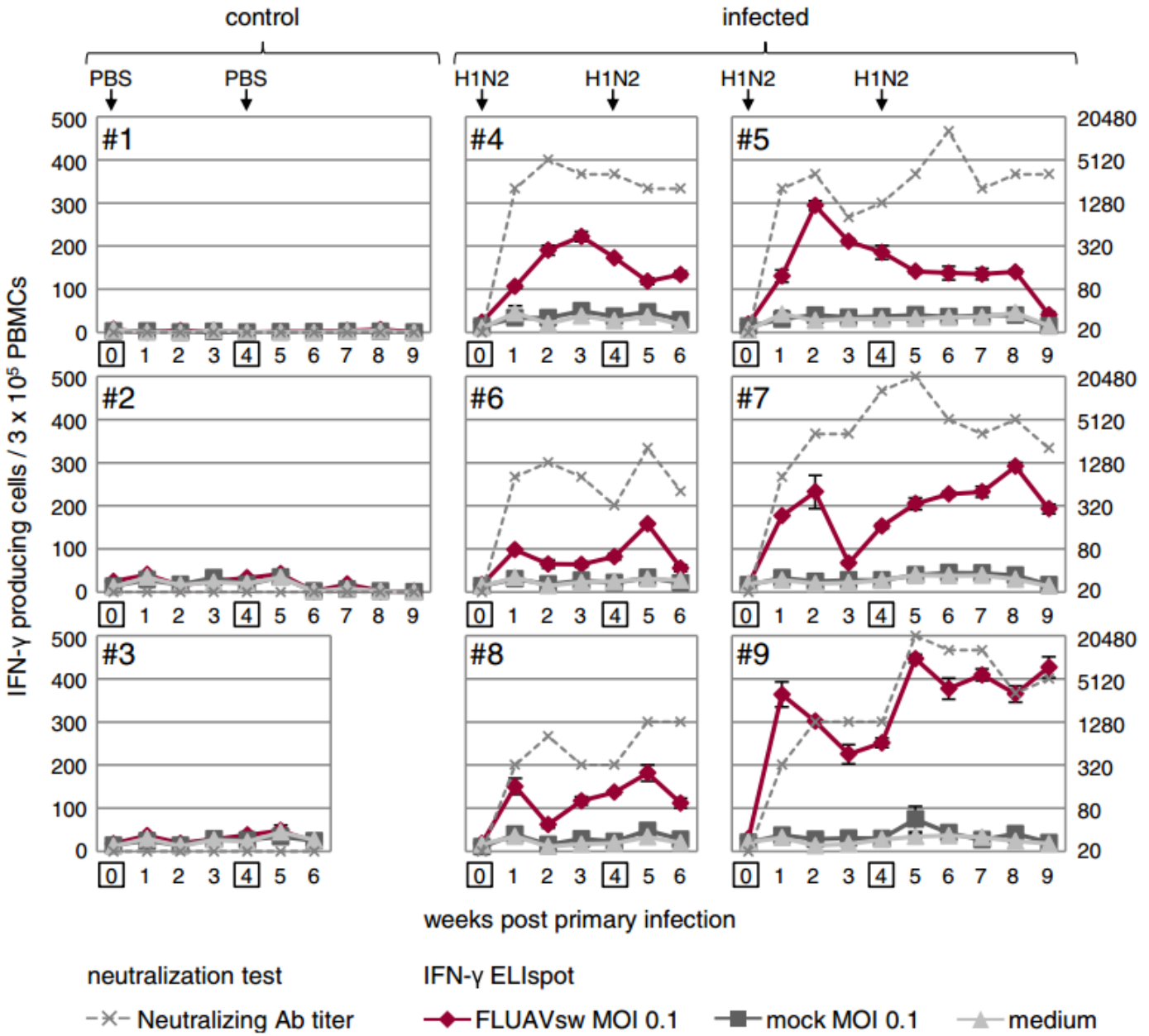
Summary of T-cell assays

Column1	equipment	high throughput	time	Identification of subset	function	frequency	labour
3H thymidine	harvester & counter	+++	5-6 d	no	no	no	+
CFSE, VS	FCM	+	5-7 d	yes	no	no	++
Activation markers	FCM	+	1-3 d	yes	no	no	++
ELISPOT	ELISPOT reader	++	2-7 d	no	yes	yes	+
ELISA	ELISA reader	+++	2-4 d	no	yes	no	+
RT-PCR	Real time PCR	++	2-4 d	no	yes	no	++
IC cytokines	FCM	+	2 d	yes	yes	yes	++
CTL assay	counter/FCM	-	7-10 d	no	yes	no	+++
CD107 assay	FCM	+	2 d	yes	yes	yes	++

CoP for influenza vaccines in humans

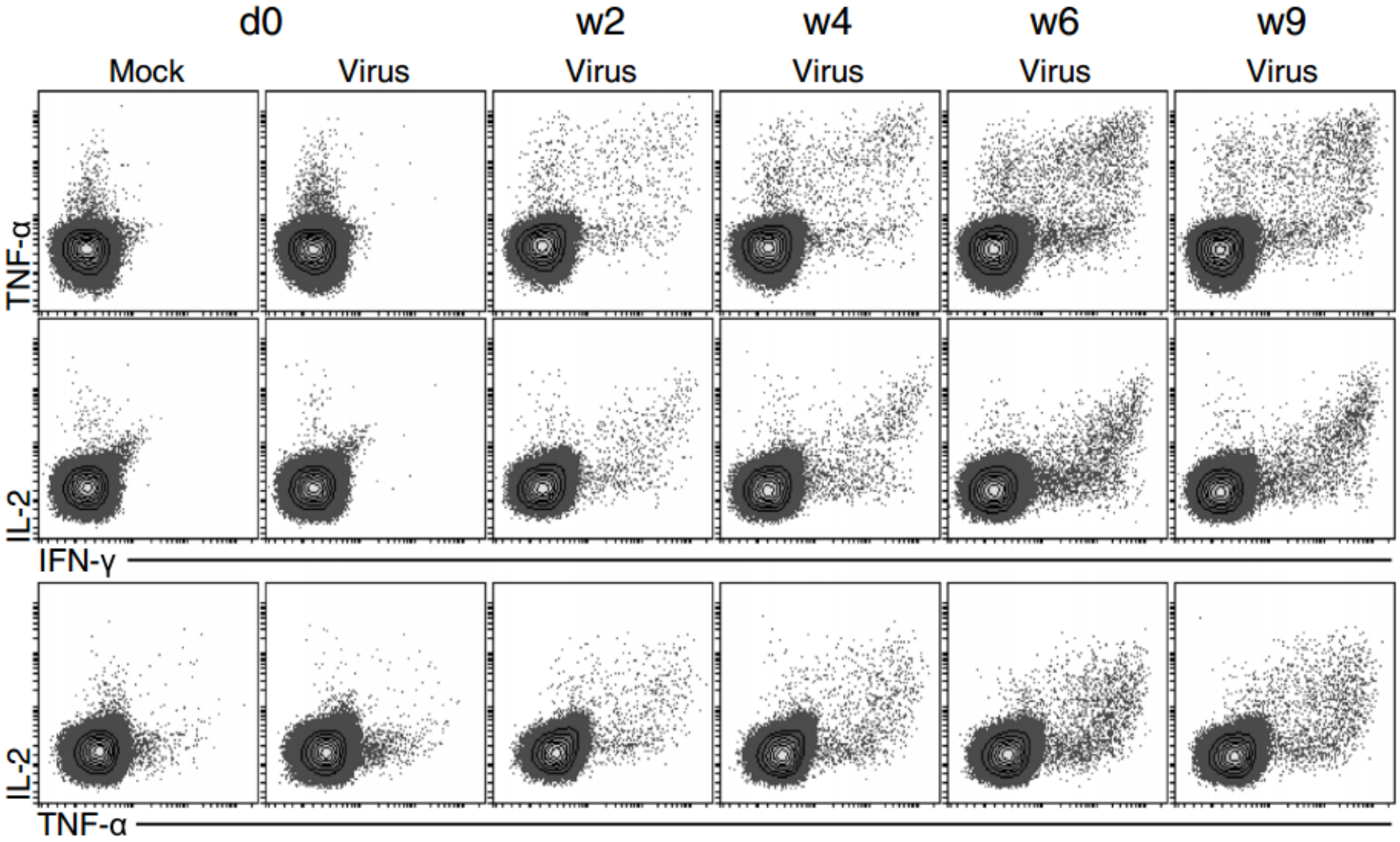
	Inactivated Influenza Vaccine	Live Attenuated Influenza Vaccine
HAI response	+++	+
Antibody secreting cells	++	+
Memory B cells	+	+
Nasal IgA	−/+	+++
NA antibody	−/+	++
CD4 T cells	++	+++
CD8 T cells	−	+?
Cross protective immunity	−/+	++

IFN-γ ELISPOT response to influenza virus infection



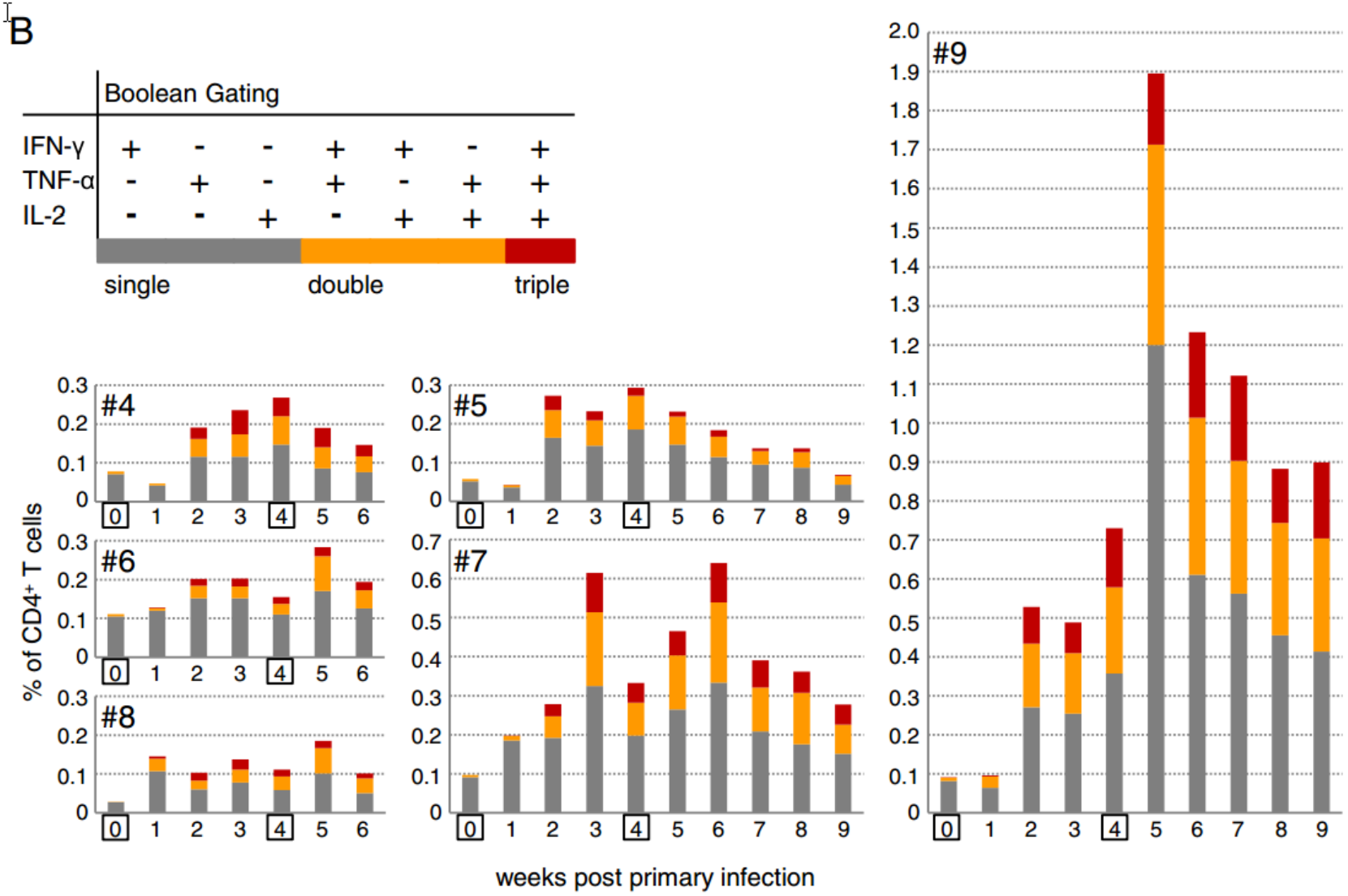
Talker et al., 2015, Vet Res 46:52; *DOI 10.1186/s13567-015-0182-3*

CD4 T-cell cytokine response to influenza virus infection



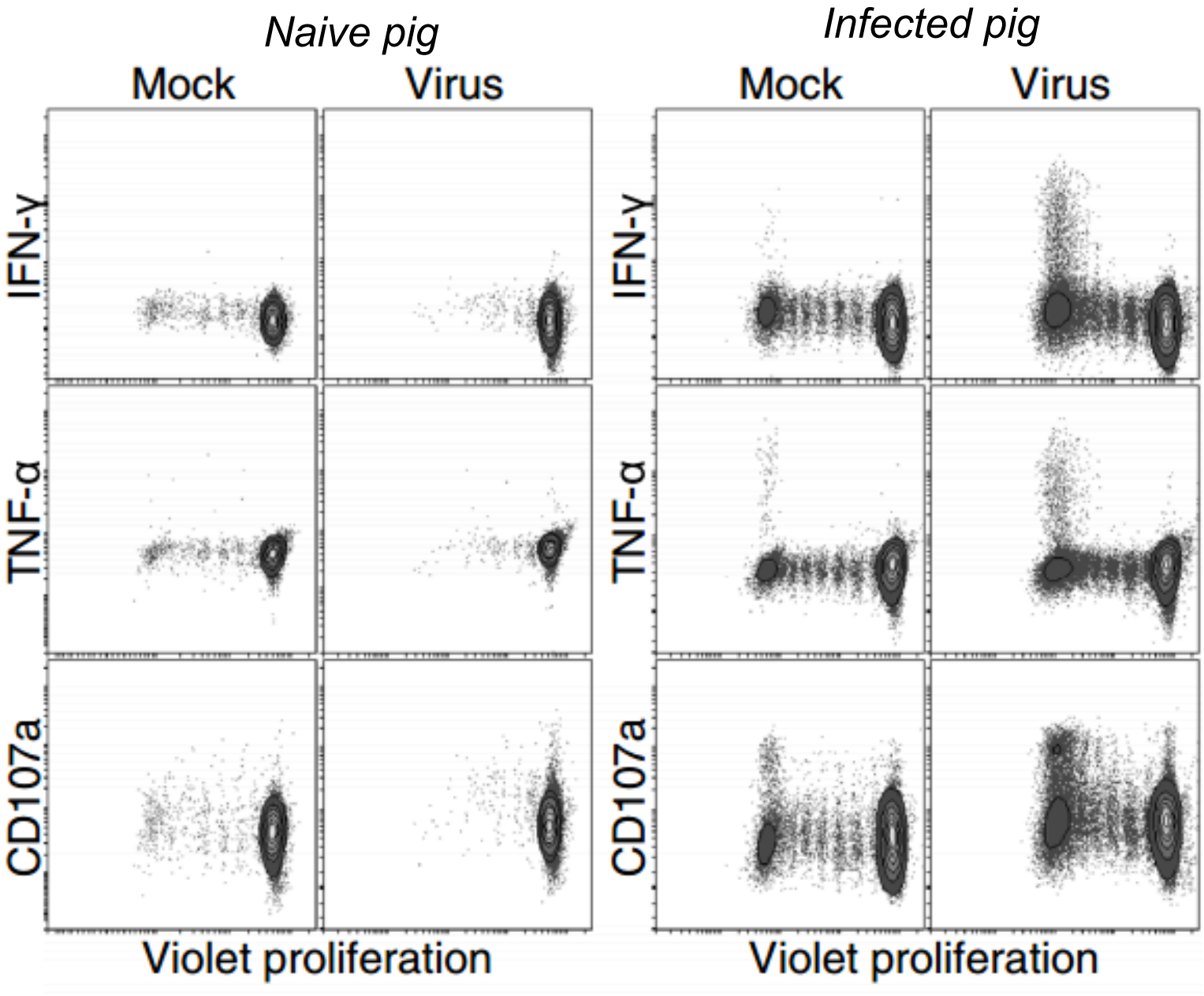
Talker et al., 2015, Vet Res 46:52; *DOI 10.1186/s13567-015-0182-3*

The porcine CD4 multifunctional T-cell response to influenza virus

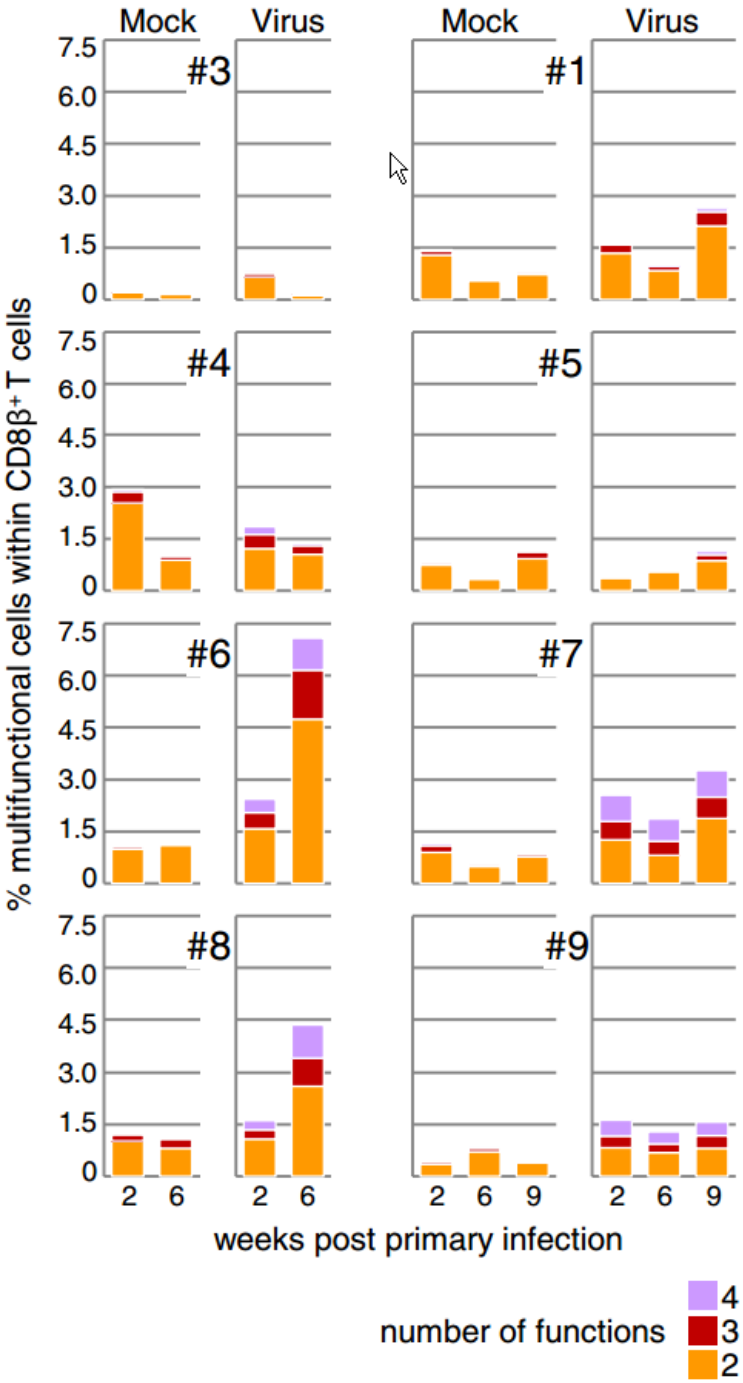


Talker et al., 2015, Vet Res 46:52; *DOI 10.1186/s13567-015-0182-3*

Multifunctional CD8 T-cell response to influenza virus



Talker et al., 2015, Vet Res 46:52



Naive pigs

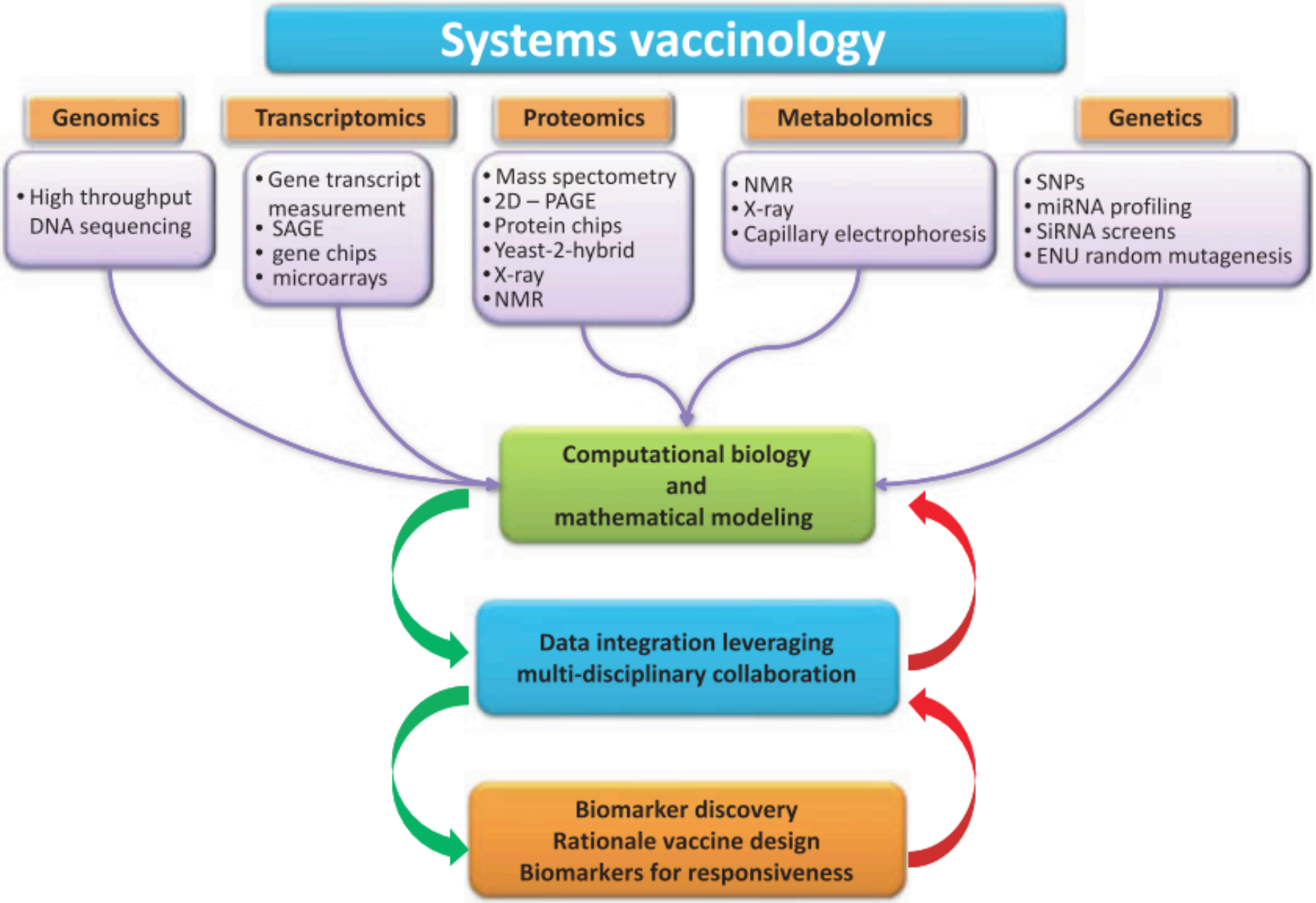
Infected pigs

Infected pigs

Infected pigs

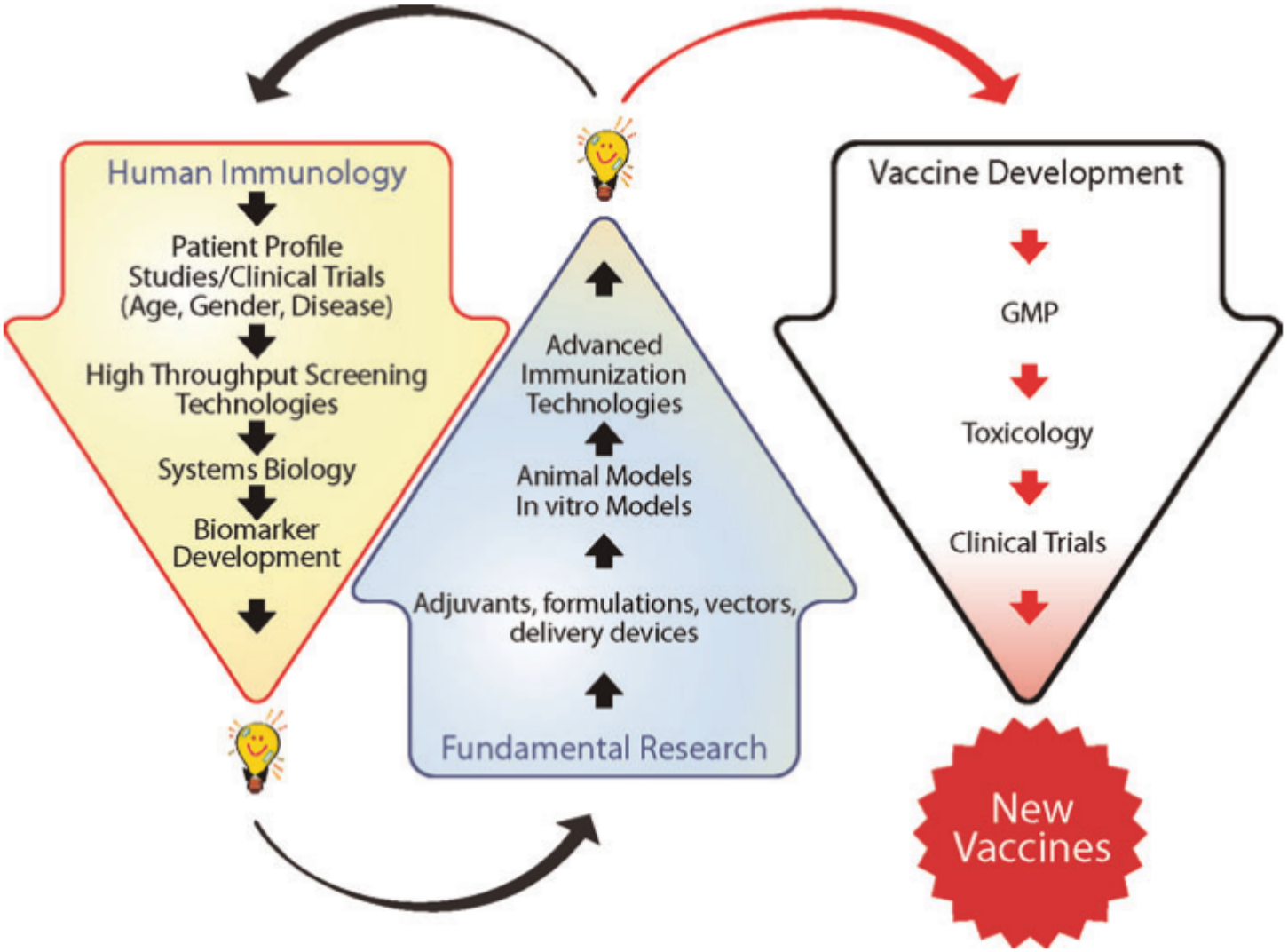
Multifunctional CD8 T-cell response to influenza virus

Talker et al., 2015, Vet Res 46:52



Buonaguro & Pulendran, Immunogenomics and systems vaccinology, Immunol. Rev. 2011; 239: 197.

Flow activities in vaccinomics



Bernstein et al., OMICS. 2011. 15: 529

How did all get started?

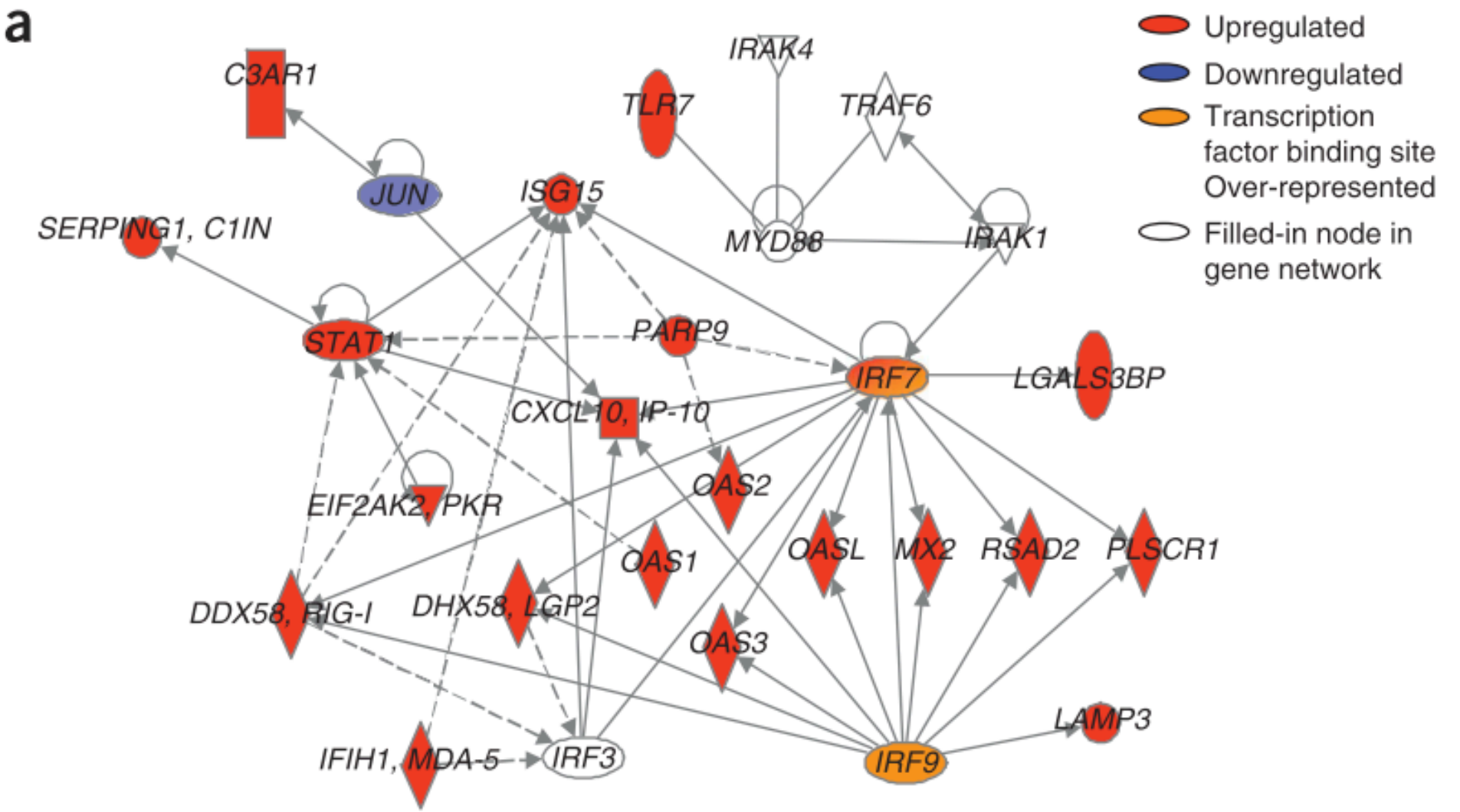
Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans

Troy D Querec[1,8], Rama S Akondy[1,8], Eva K Lee[2], Weiping Cao[1], Helder I Nakaya[1], Dirk Teuwen[3], Ali Pirani[4], Kim Gernert[4], Jiusheng Deng[1], Bruz Marzolf[5], Kathleen Kennedy[5], Haiyan Wu[5], Soumaya Bennouna[1], Herold Oluoch[1], Joseph Miller[1], Ricardo Z Vencio[5], Mark Mulligan[1,6], Alan Aderem[5], Rafi Ahmed[1] & Bali Pulendran[1,7]

A major challenge in vaccinology is to prospectively determine vaccine efficacy. Here we have used a systems biology approach to identify early gene 'signatures' that predicted immune responses in humans vaccinated with yellow fever vaccine YF-17D. Vaccination induced genes that regulate virus innate sensing and type I interferon production. Computational analyses identified a gene signature, including complement protein C1qB and eukaryotic translation initiation factor 2 alpha kinase 4—an orchestrator of the integrated stress response—that correlated with and predicted YF-17D CD8+ T cell responses with up to 90% accuracy in an independent, blinded trial. A distinct signature, including B cell growth factor *TNFRS17*, predicted the neutralizing antibody response with up to 100% accuracy. These data highlight the utility of systems biology approaches in predicting vaccine efficacy.

Querec et al, Nat Immunol 2009; doi:10.1038/ni.1688

Pathway analysis reveals many genes of the IFN pathways being upegulated



Querec et al, Nat Immunol 2009; doi:10.1038/ni.1688

List of genes correlating with CD8 T cells responses

Gene name	Gene symbol	Gene ID
Solute carrier family 2 (facilitated glucose transporter), member 6	*SLC2A6*	Hs.244378 Day 7
Eukaryotic translation initiation factor 2 alpha kinase 4	*EIF2AK4*	Hs.412102 Day 7
Integrin, alpha L (antigen CD11A)	*ITGAL/LFA-1*	Hs.174103 Day 7
C-terminal binding protein 1	*CTBP1*	Hs.208597 Day 7
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	*YWHAE*	Hs.513851 Day 3
Transcribed locus		Hs.619443 Day 7
Protein phosphatase 1, regulatory (inhibitor) subunit 14A	*PPP1R14A*	Hs.631569 Day 3
Family with sequence similarity 62 member B	*FAM62B*	Hs.649908 Day 7
Transcribed locus		Hs.42650 Day 7

Querec et al, Nat Immunol 2009; doi:10.1038/ni.1688

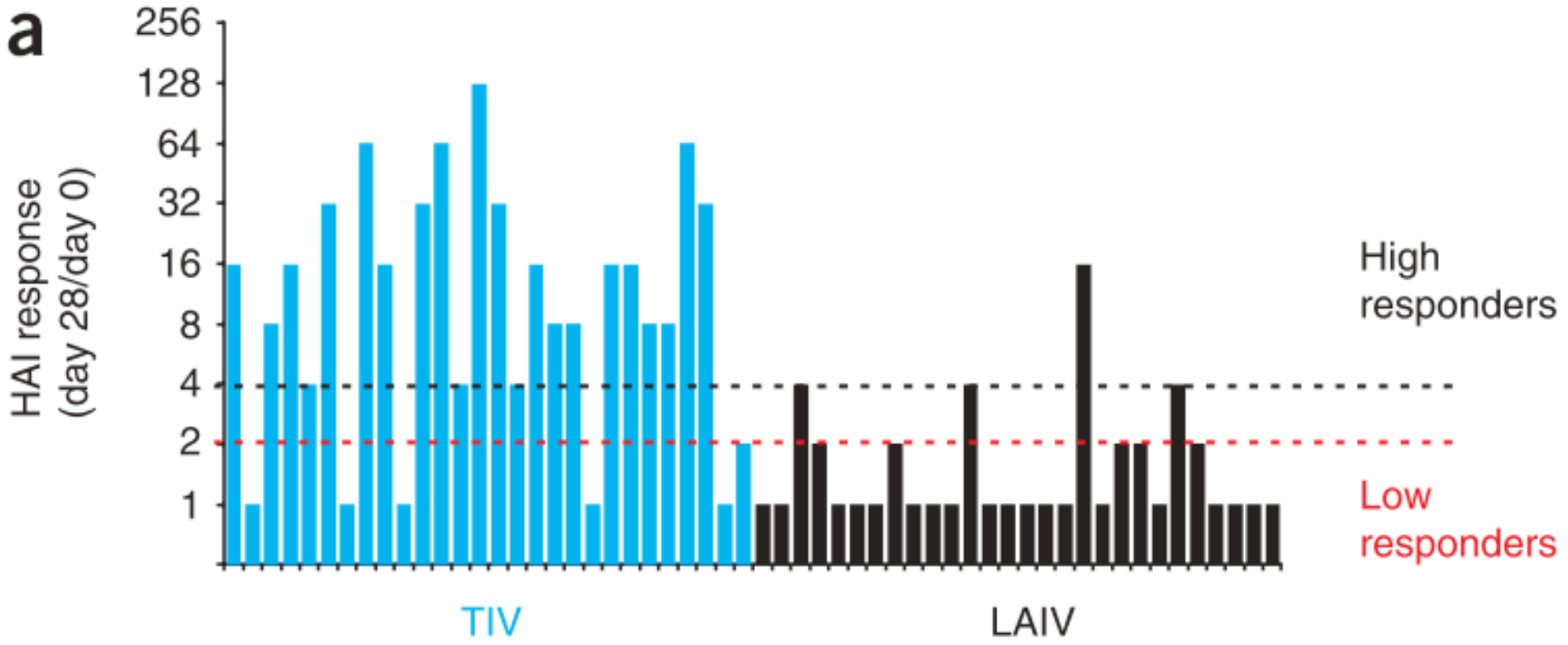
List of genes correlating with neutralizing antibody responses

Gene name	Gene symbol	Gene ID
BEN domain–containing 4	*BEND4*	Hs.120591
Transcribed locus		Hs.139006
6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	*PFKFB3*	Hs.195471
Tumor necrosis factor receptor superfamily, member 17	*TNFRSF17*	Hs.2556
Tumor protein D52	*TPD52*	Hs.368433
Transcribed locus		Hs.481166
Kelch repeat and BTB (POZ) domain containing 7	*KBTBD7*	Hs.63841
Transcribed locus		Hs.649726
Nucleosome assembly protein 1-like 2	*NAP1L2*	Hs.66180

Querec et al, Nat Immunol 2009; doi:10.1038/ni.1688

Systems biology of vaccination for seasonal influenza in humans

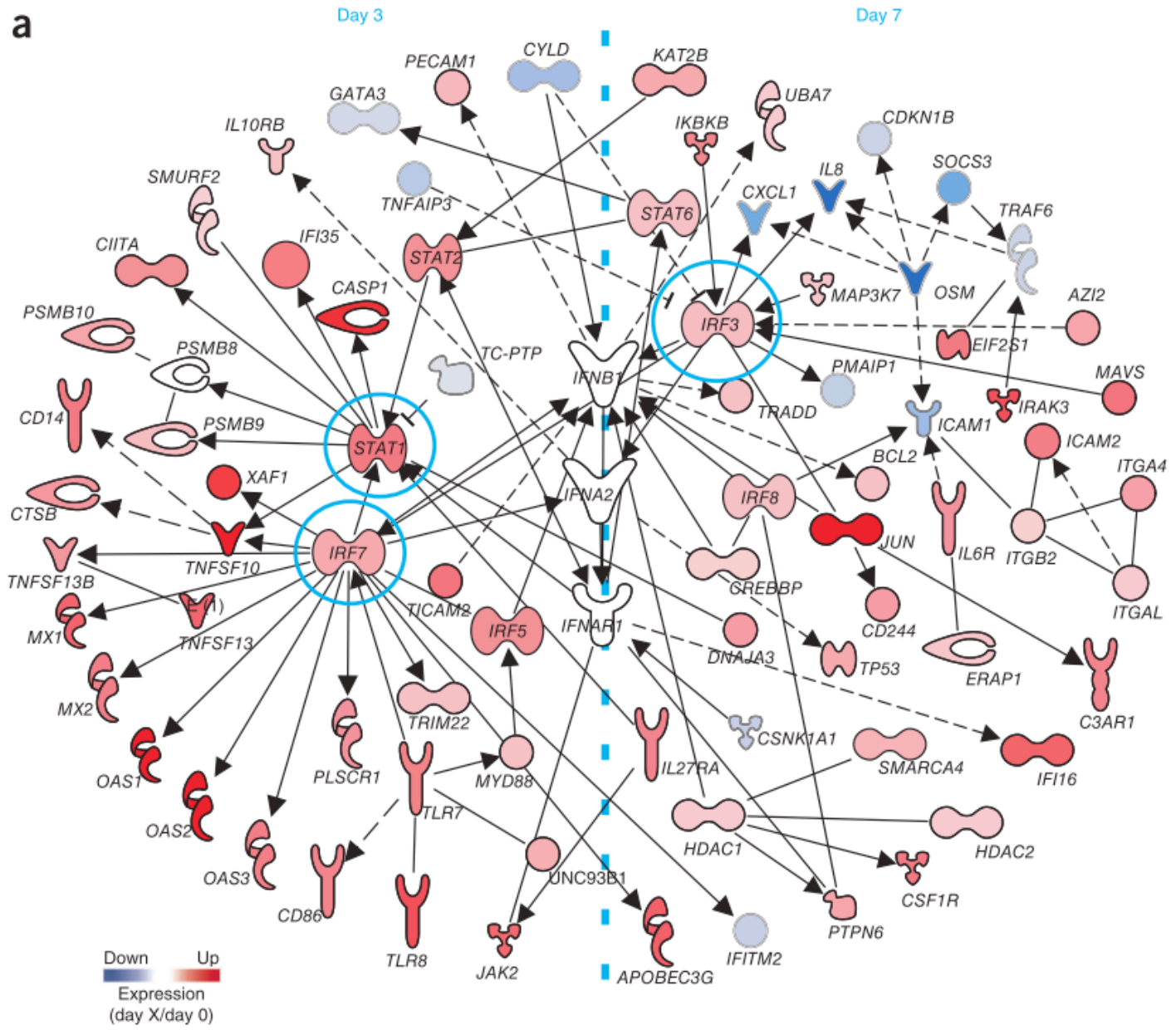
Helder I Nakaya[1,2], Jens Wrammert[1,3], Eva K Lee[4], Luigi Racioppi[5,6], Stephanie Marie-Kunze[1,2], W Nicholas Haining[7], Anthony R Means[6], Sudhir P Kasturi[1,2], Nooruddin Khan[1,2], Gui-Mei Li[1,3], Megan McCausland[1,3], Vibhu Kanchan[1,3], Kenneth E Kokko[8], Shuzhao Li[1,2], Rivka Elbein[9], Aneesh K Mehta[9], Alan Aderem[10], Kanta Subbarao[11], Rafi Ahmed[1,3] & Bali Pulendran[1,2,12]



Nakaya et al, Nat Immunol 2011; doi:10.1038/ni.2067

What do you expect will be the main difference between the LIAV and TIV vaccine?

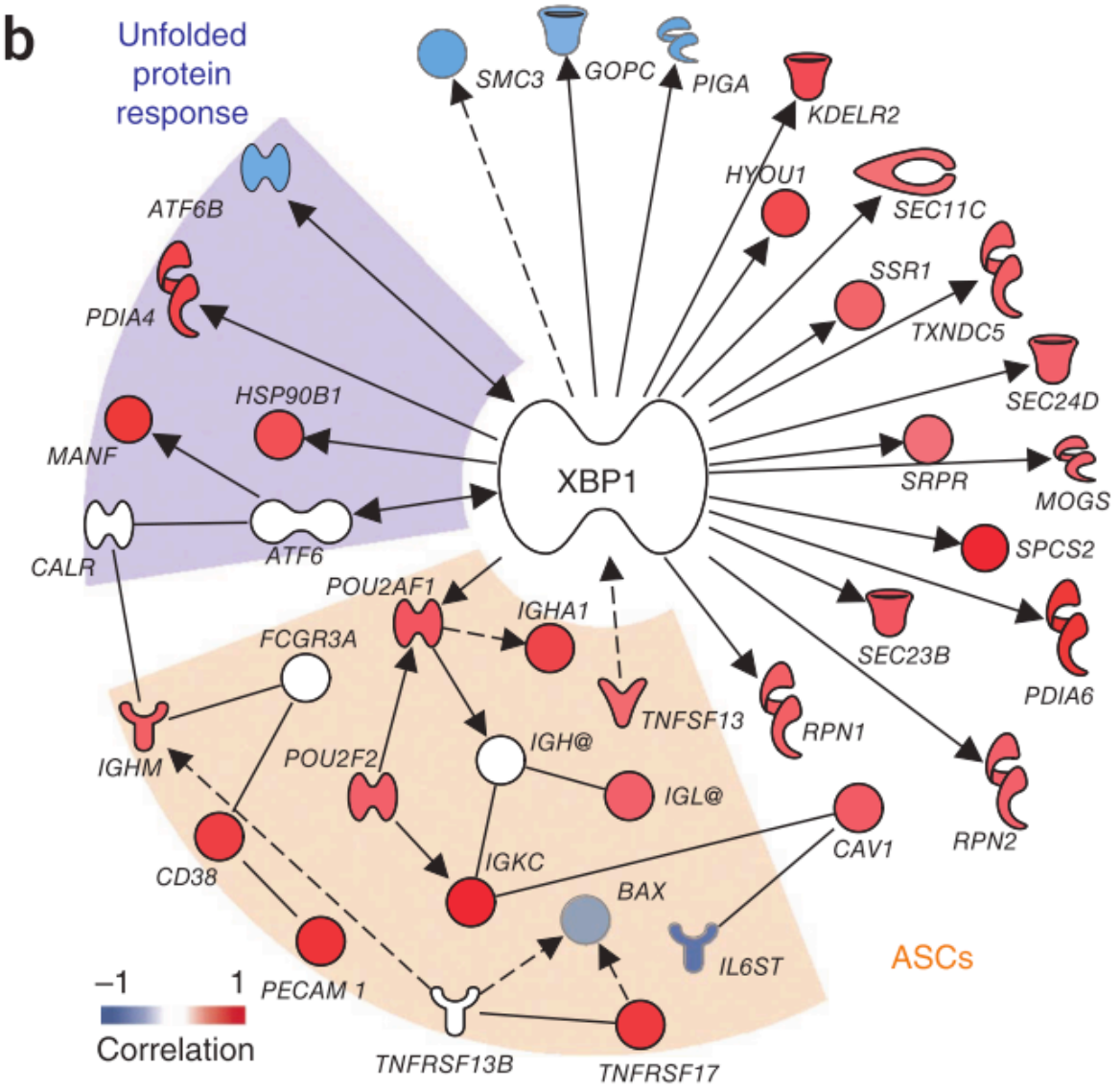
LAIV response is dominated by IFN-response genes



Nakaya et al, Nat Immunol 2011; doi:10.1038/ni.2067

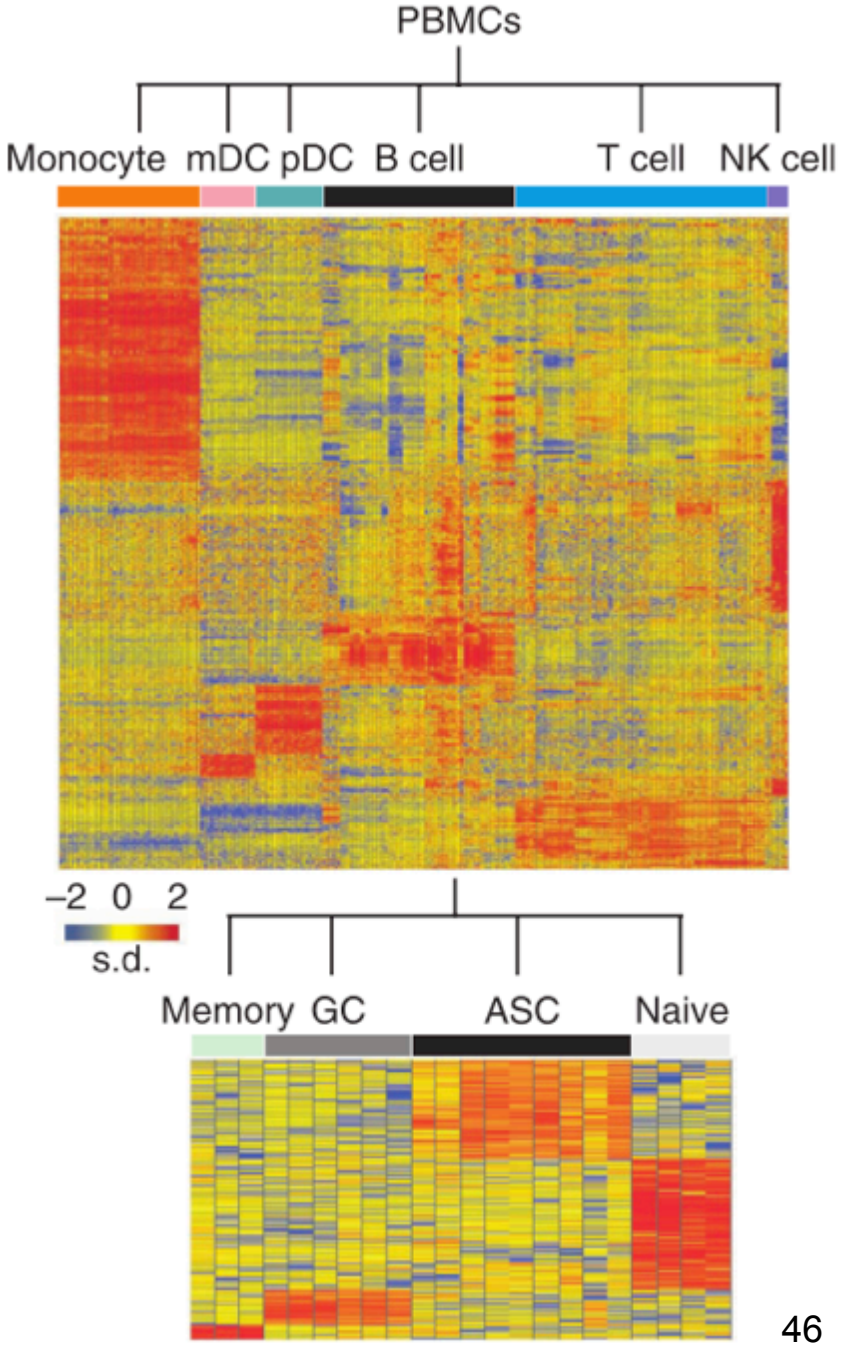
Molecular signatures that correlate with titers of antibody to TIV

Unfolded protein response:
helps coping with the large amount of Ig produced in ASCs which is associated with accumulation of misfolded proteins.
→ Enhanced secretion
→ XBP1 is central



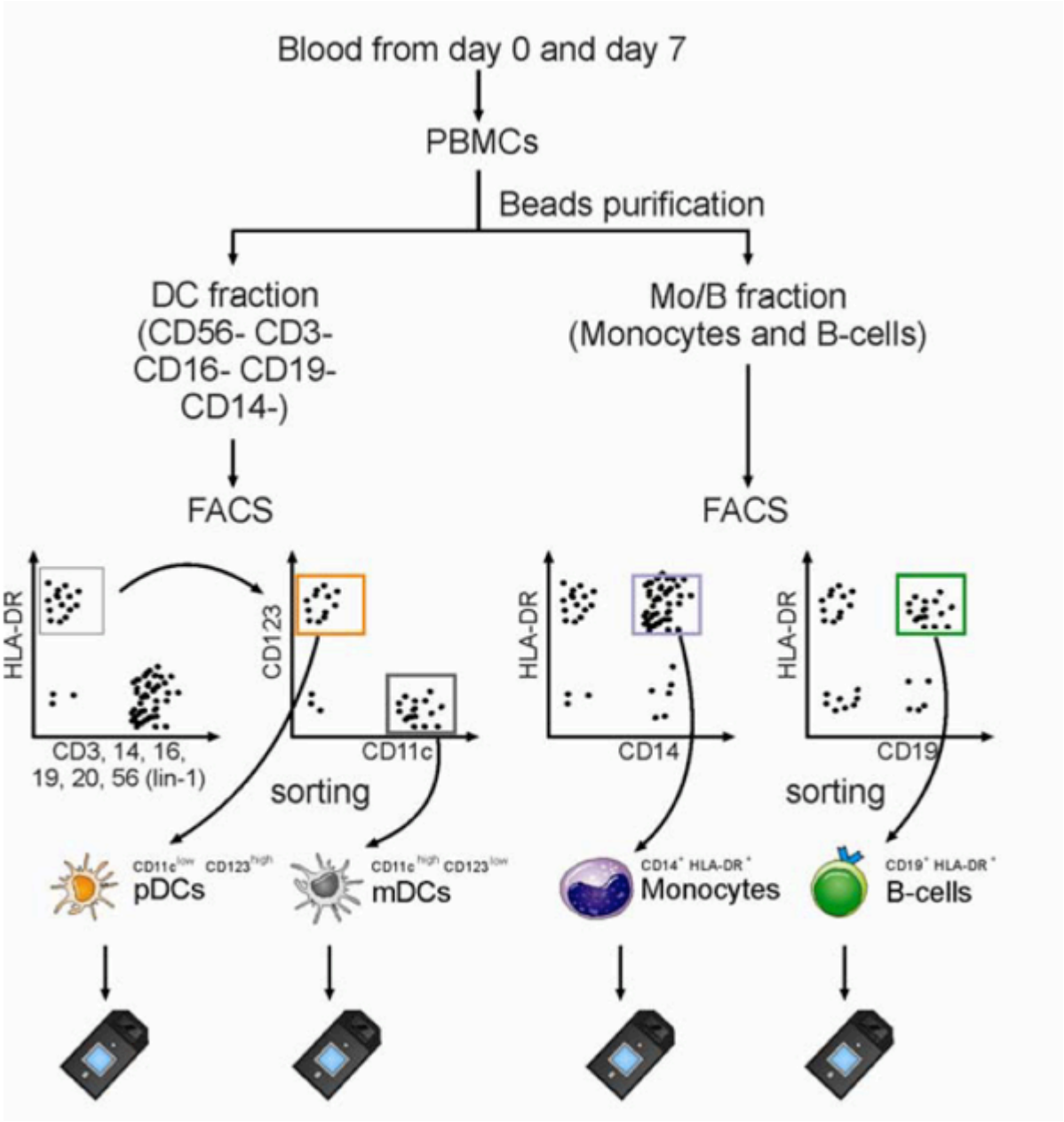
ASC response

Heatmap of gene signatures of cells of the human immune system



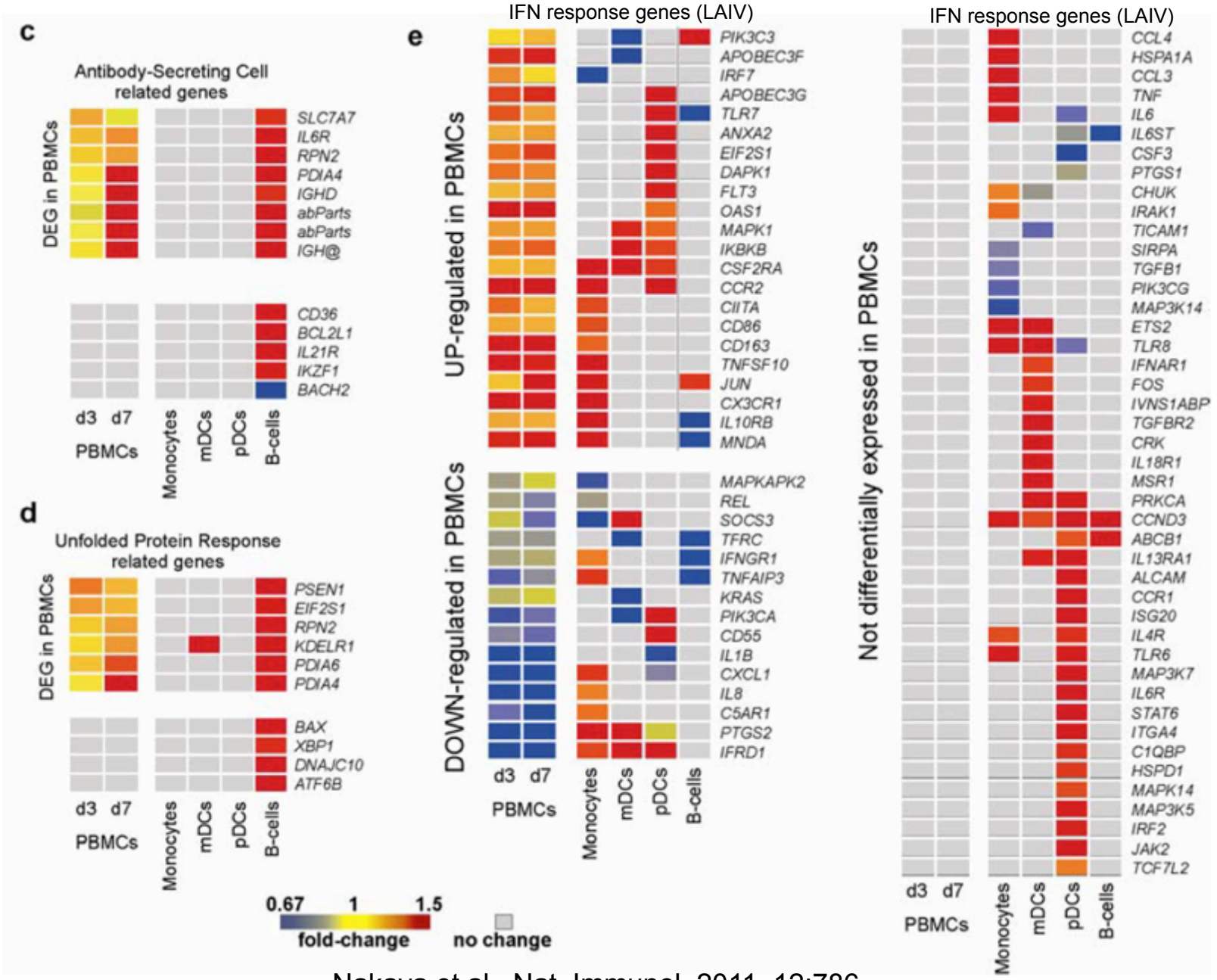
Nakaya et al, Nat Immunol 2011; doi:10.1038/ni.2067

Gene expression in sorted cells following vaccination with LAIV



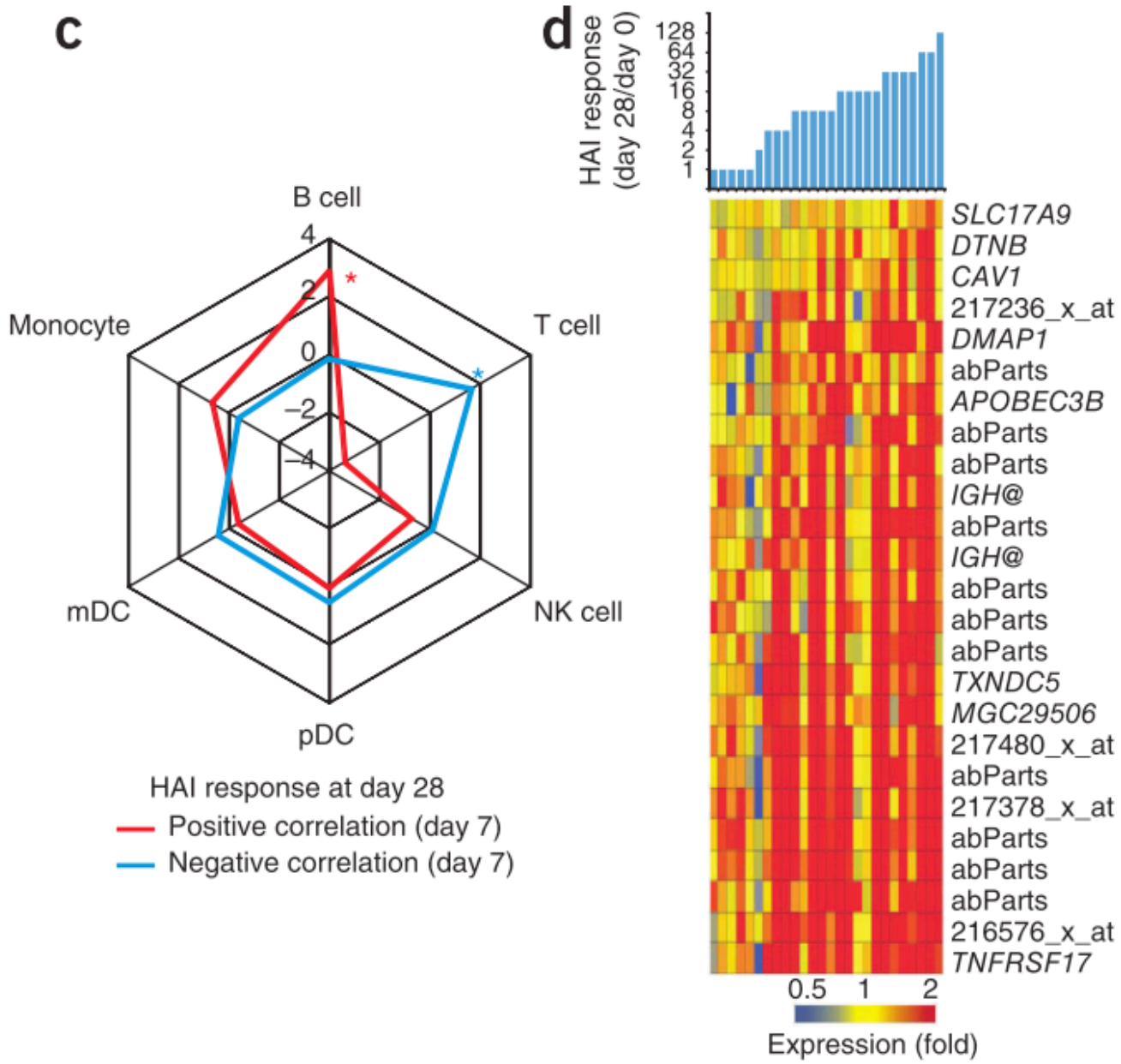
Nakaya et al., Nat. Immunol. 2011, 12:786

Gene expression in sorted cells following vaccination



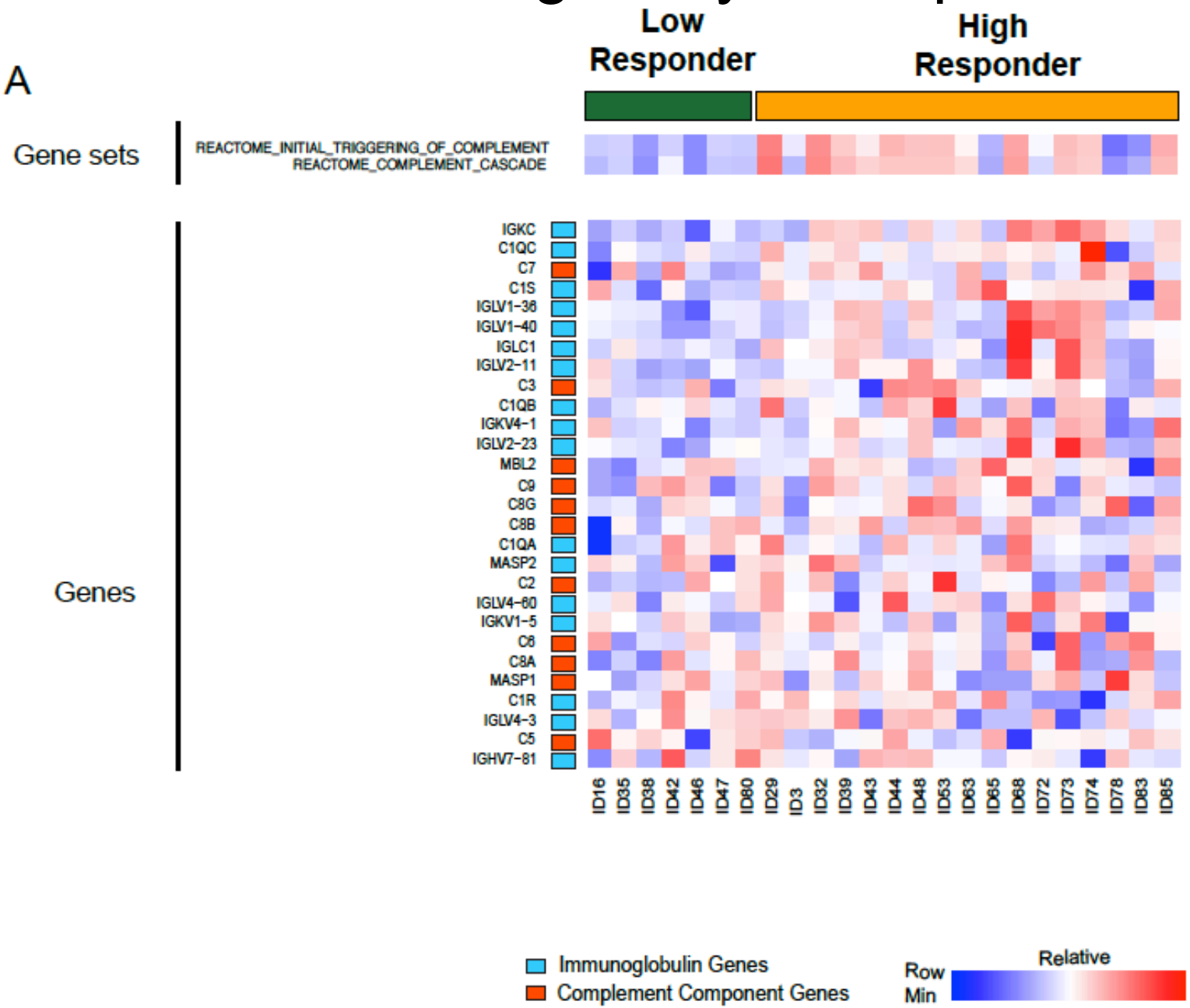
Nakaya et al., Nat. Immunol. 2011, 12:786

Molecular signatures that correlate with titers of antibody to TIV



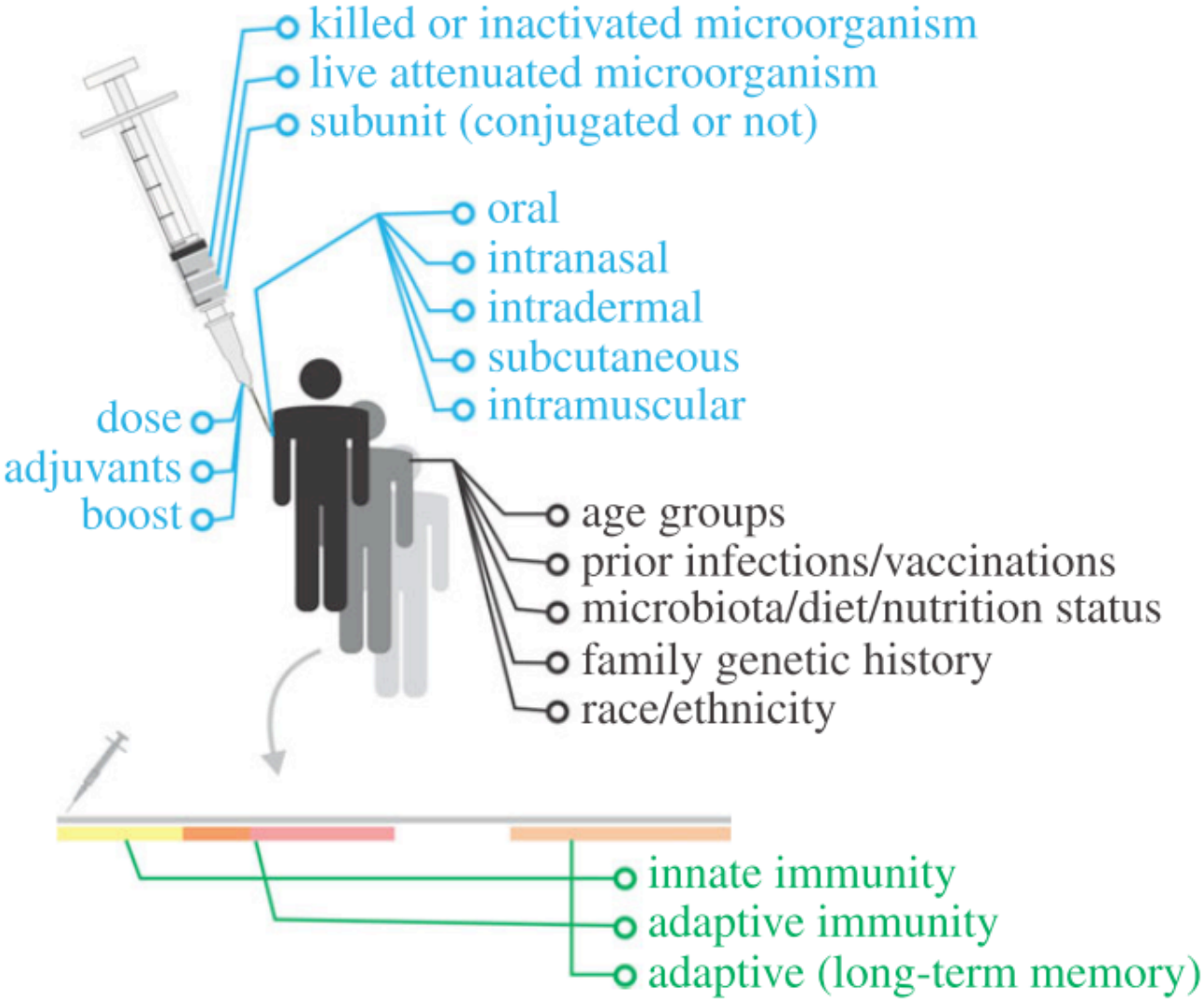
Nakaya et al., Nat. Immunol. 2011, 12:786

Problem of heterogeneity of responses



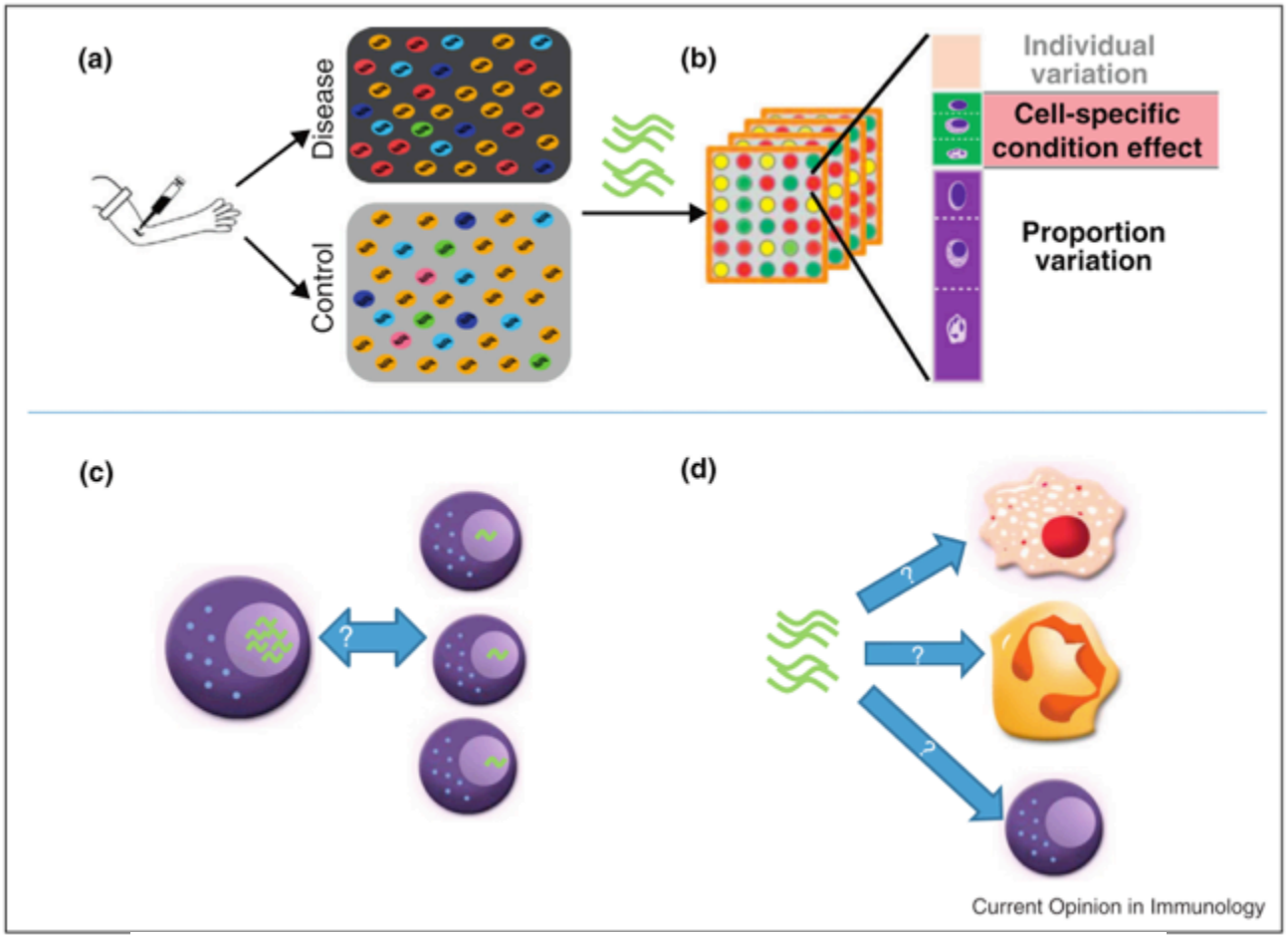
Which factors other than vaccine component will influence the transcriptomic response?

Factors influencing how a vaccine will perturb the immune system



Nakaya and Pulendran, 2015. Phil. Trans. R. Soc. B , 370(1671), 1–9.

Biological samples are heterogeneous with respect to underlying cell subsets



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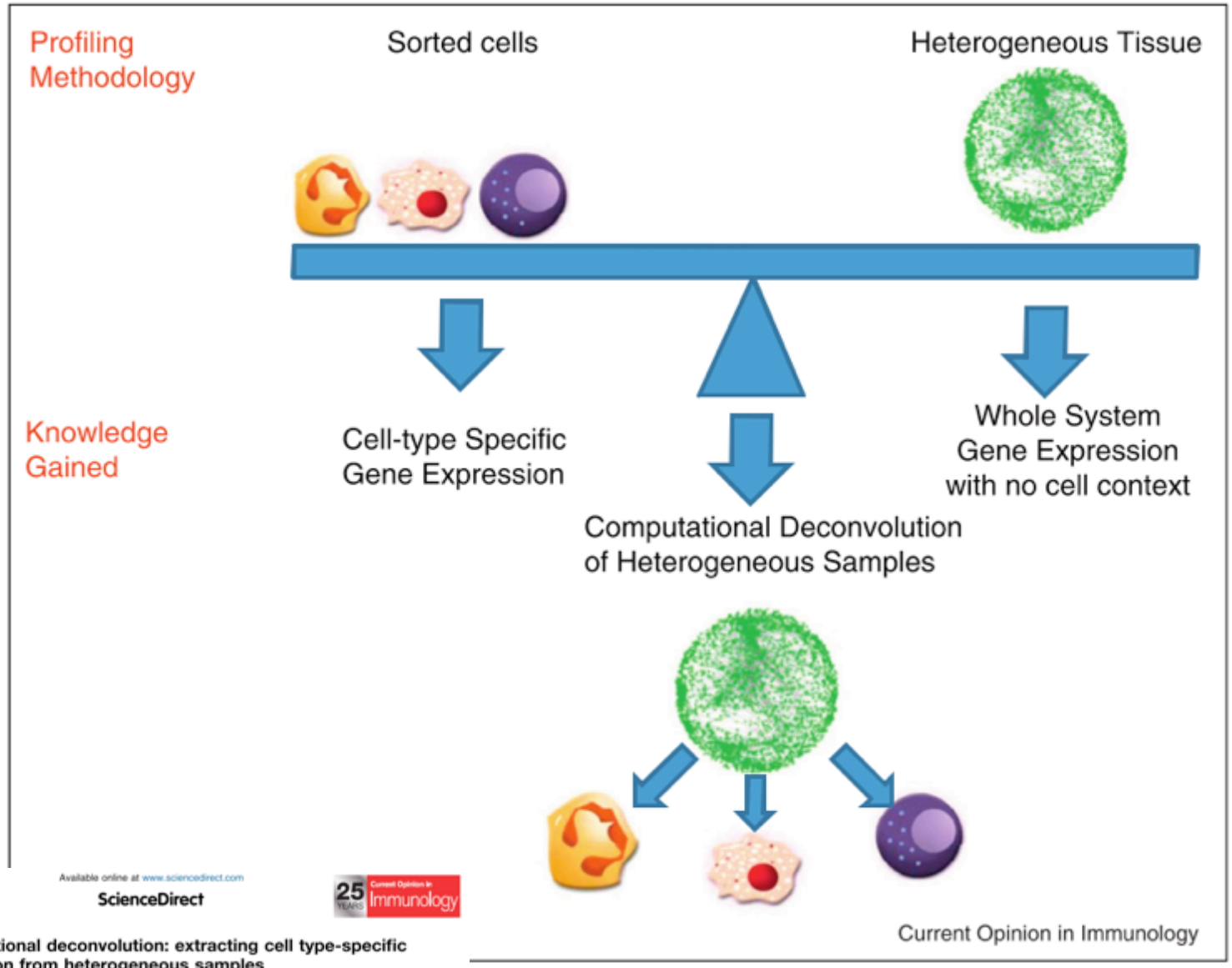
Computational deconvolution: extracting cell type-specific information from heterogeneous samples

Shai S Shen-Orr[1,2,3] and Renaud Gaujoux[2]

Computational deconvolution methodologies enable capturing both cell-centered and system



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Computational deconvolution: extracting cell type-specific information from heterogeneous samples
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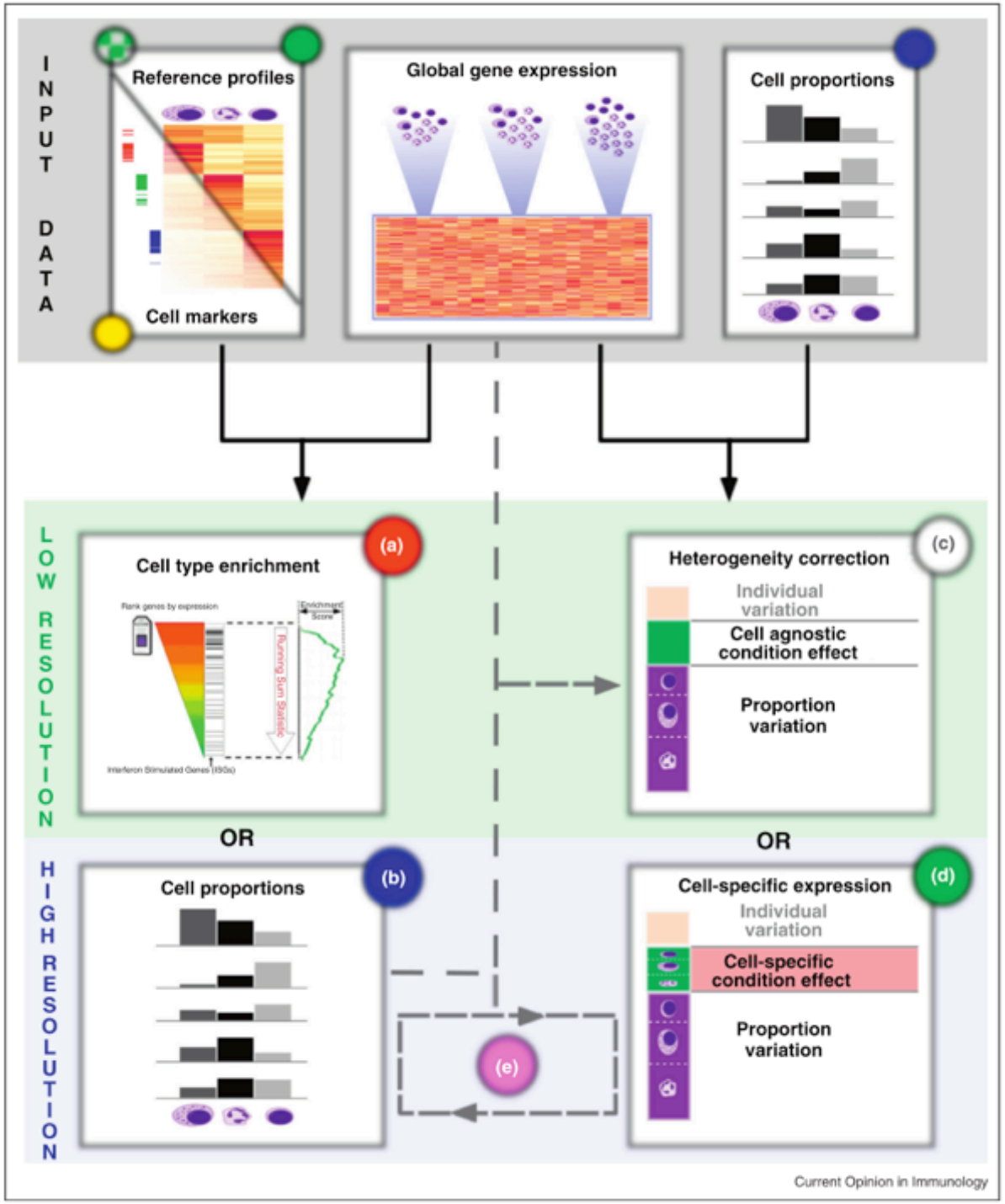
computational approaches that extract cell type-specific information from heterogeneous sample data



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Computational deconvolution: extracting cell type-specific information from heterogeneous samples
Shai S Shen-Orr[1,2,3] and Renaud Gaujoux[2]

Can we use these reference profiles in veterinary immunology?

RESOURCE

nature immunology

Molecular signatures of antibody responses derived from a systems biology study of five human vaccines

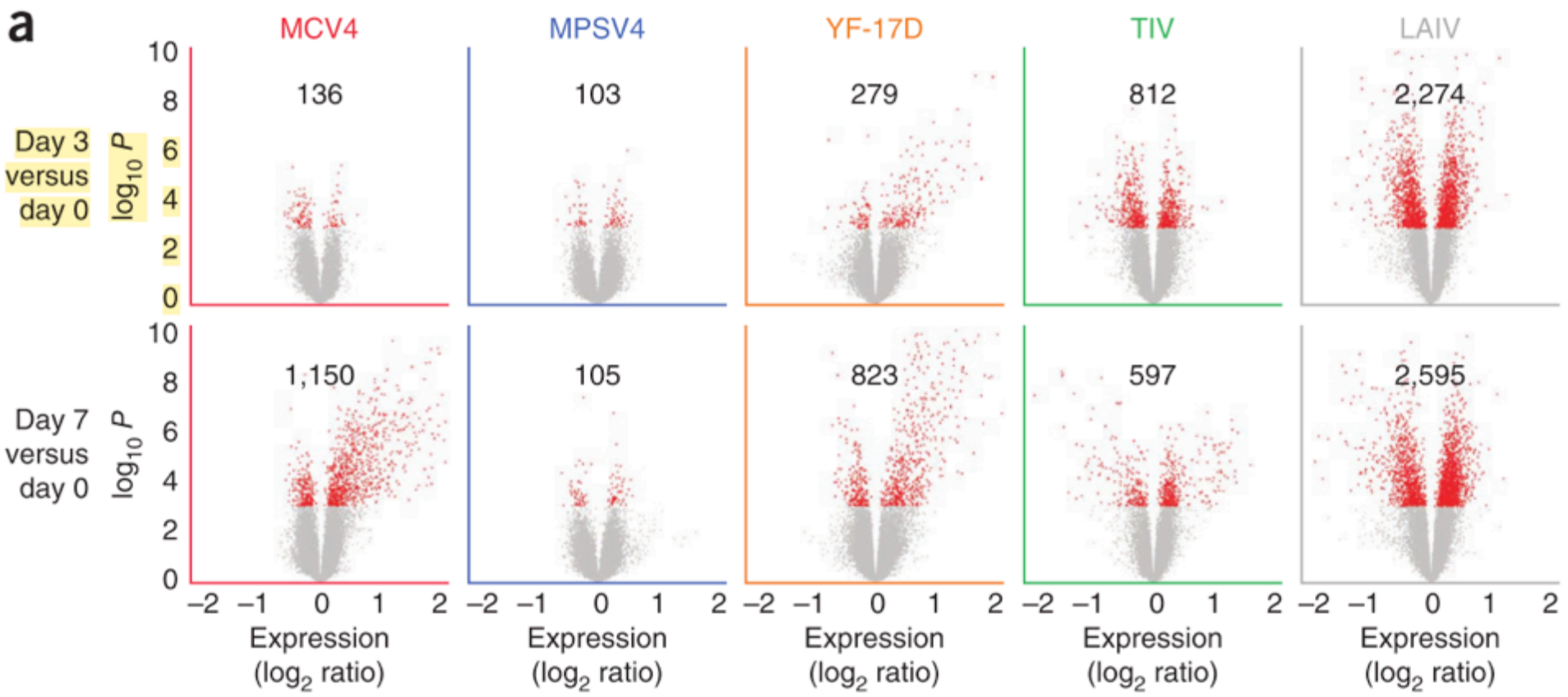
Shuzhao Li[1,2,10], Nadine Rouphael[1,3,10], Sai Duraisingham[1,2,10], Sandra Romero-Steiner[4], Scott Presnell[5,6], Carl Davis[1,7], Daniel S Schmidt[4], Scott E Johnson[4], Andrea Milton[4], Gowrisankar Rajam[4], Sudhir Kasturi[1,2], George M Carlone[4], Charlie Quinn[5,6], Damien Chaussabel[5,6], A Karolina Palucka[6], Mark J Mulligan[1,3,7], Rafi Ahmed[1,8], David S Stephens[1,7], Helder I Nakaya[1,2,9] & Bali Pulendran[1,2,9]

Li et al., 2014. Nat Immunol **15**, 195

Meningoccus vaccines

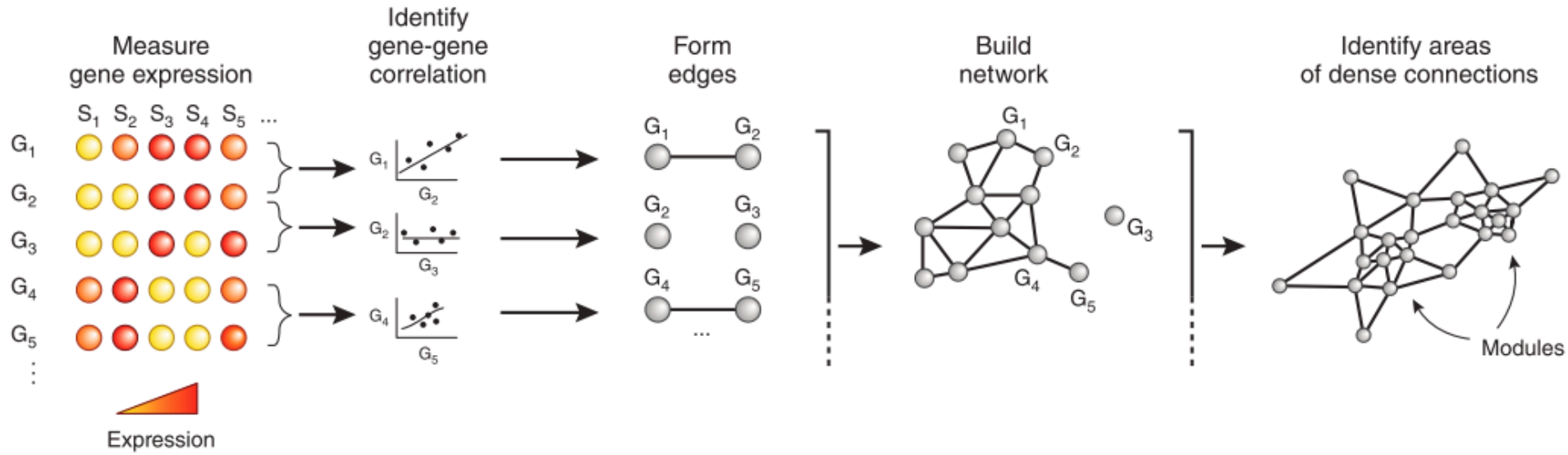
PS conjugated to DT

YFV live **Flu killed** **Flu live**



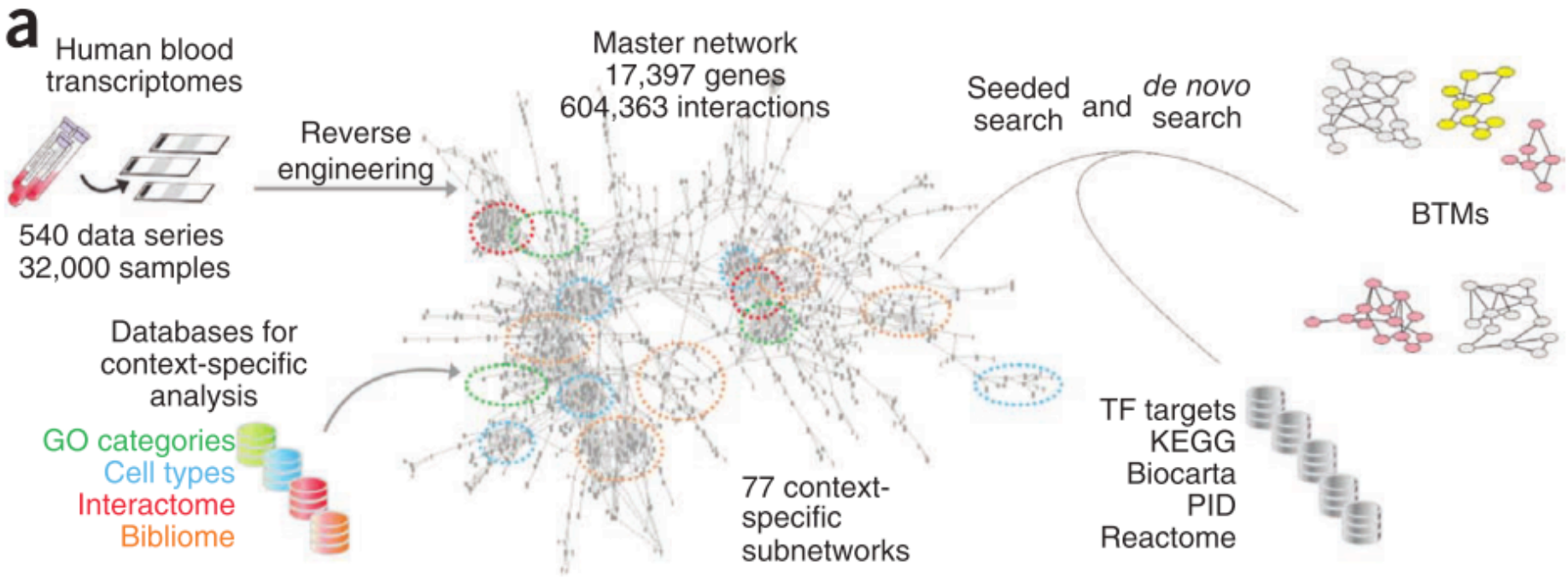
Li et al., 2014. Nat Immunol **15**, 195

“Strength in numbers: comparing vaccine signatures the modular way”



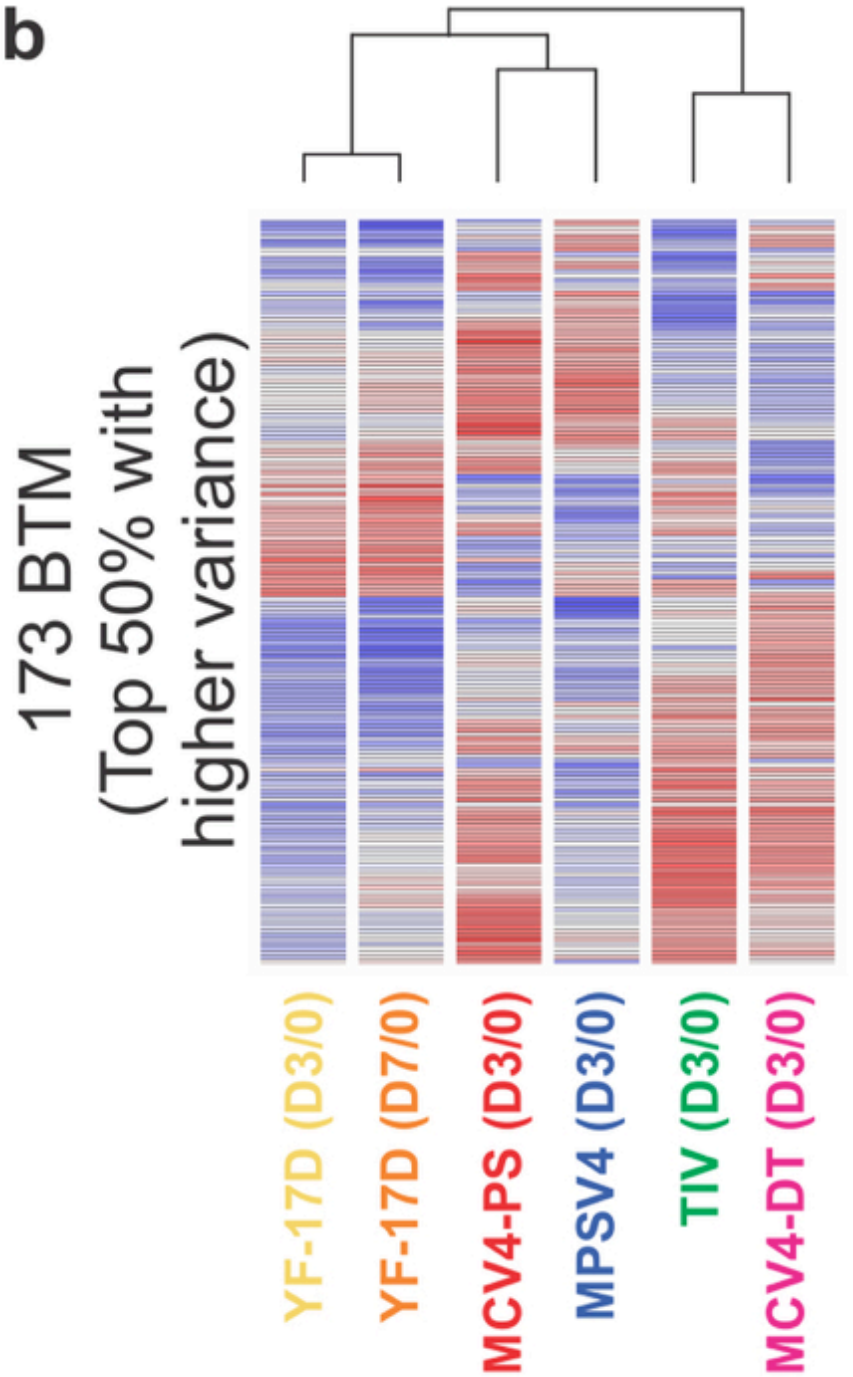
Haining, Nat. Immunol 2014; 15: 139

Construction of blood transcriptional modules (BTMs) through large-scale data integration



Li et al., 2014. Nat Immunol **15**, 195

Heat map of BTMs (rows) and vaccines (columns) whose expression correlated with antibody response.

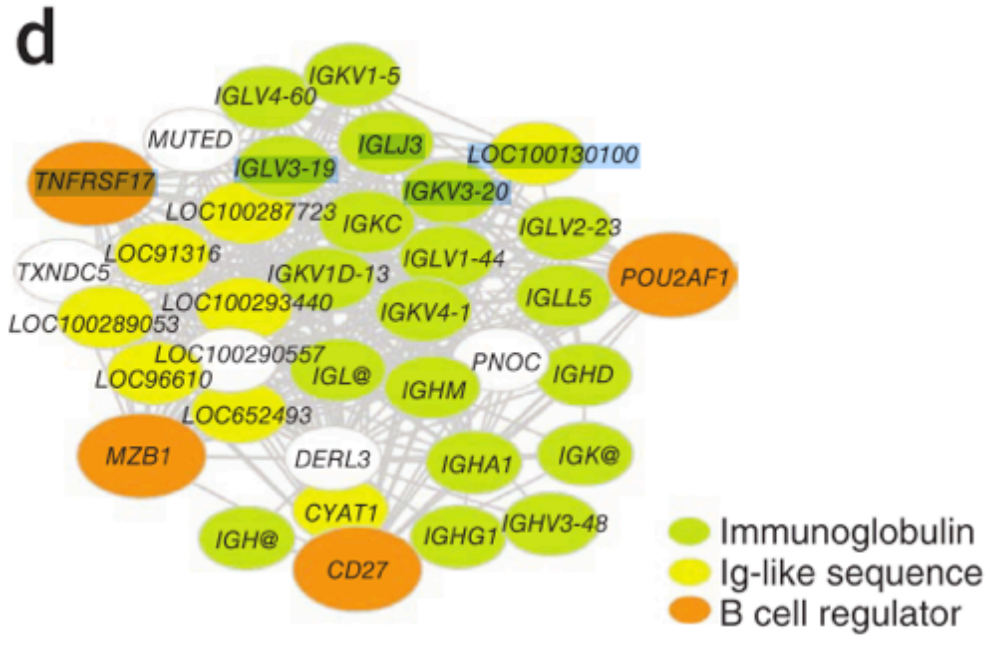


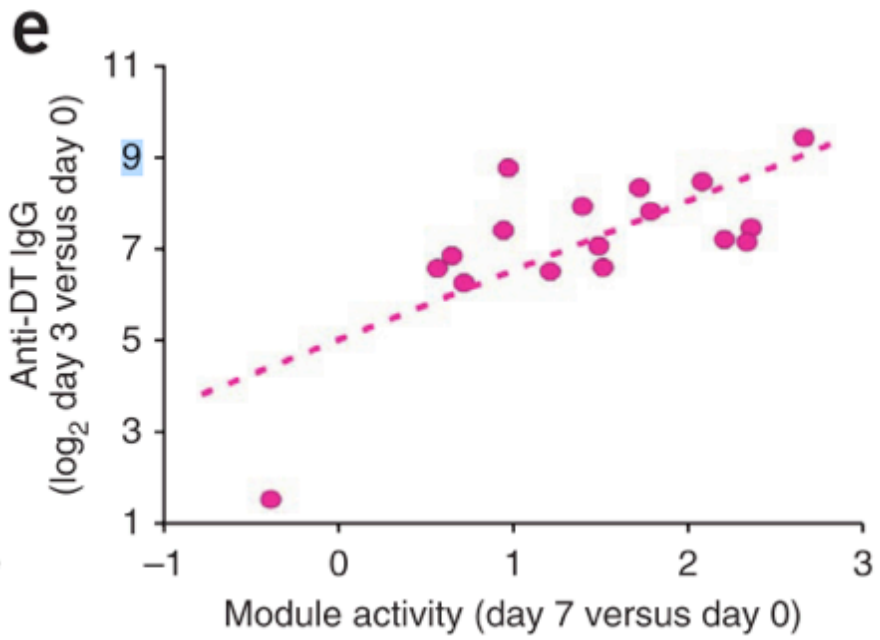
Li et al., 2014. Nat Immunol **15**, 195

BTM correlation profiles displayed three distinct patterns:

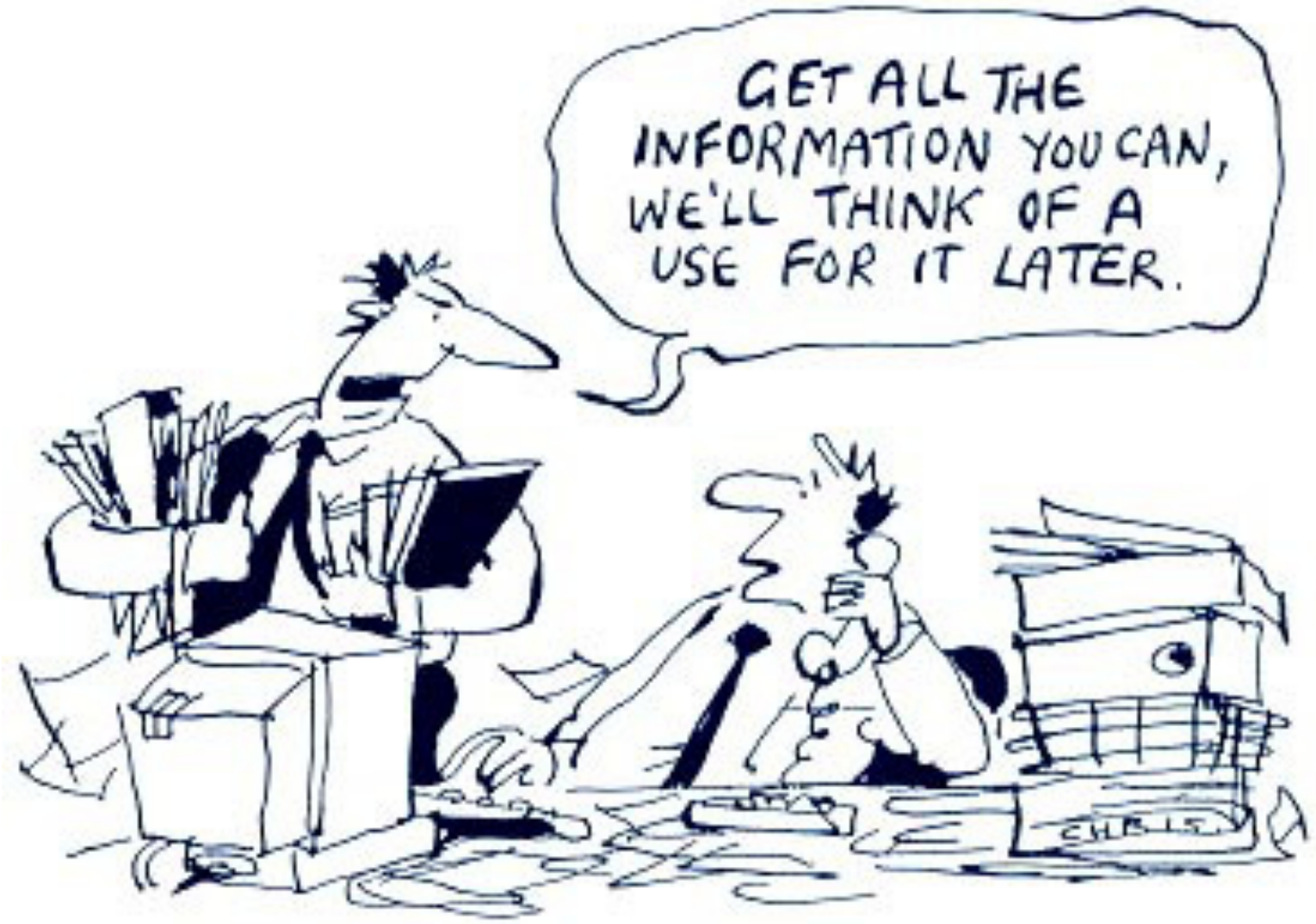
1. protein recall response (TIV, MCV4-DT),
2. polysaccharide response (MPSV4, MCV4)
3. primary viral response (YF-17D).

BTM M156.1 correlates with specific antibodies (MCV4 vaccine) day 7 p.v.





Li et al., 2014. Nat Immunol **15**, 195



System vaccinology in veterinary vaccinology