STANFORD
SCHOOL OF MEDICINE
Stanford University Medical Center

Systems Biology Approaches to Influenza Vaccination

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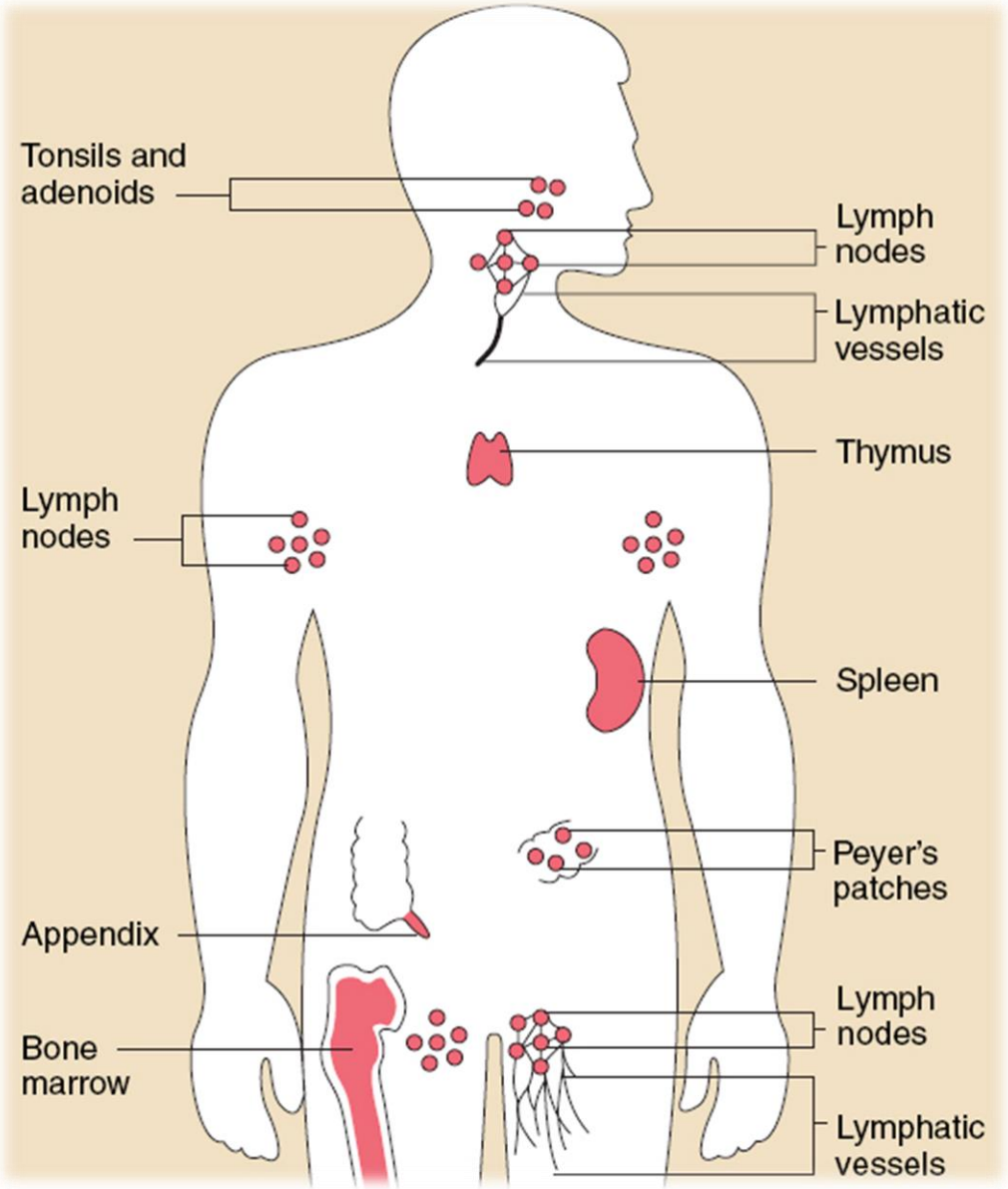
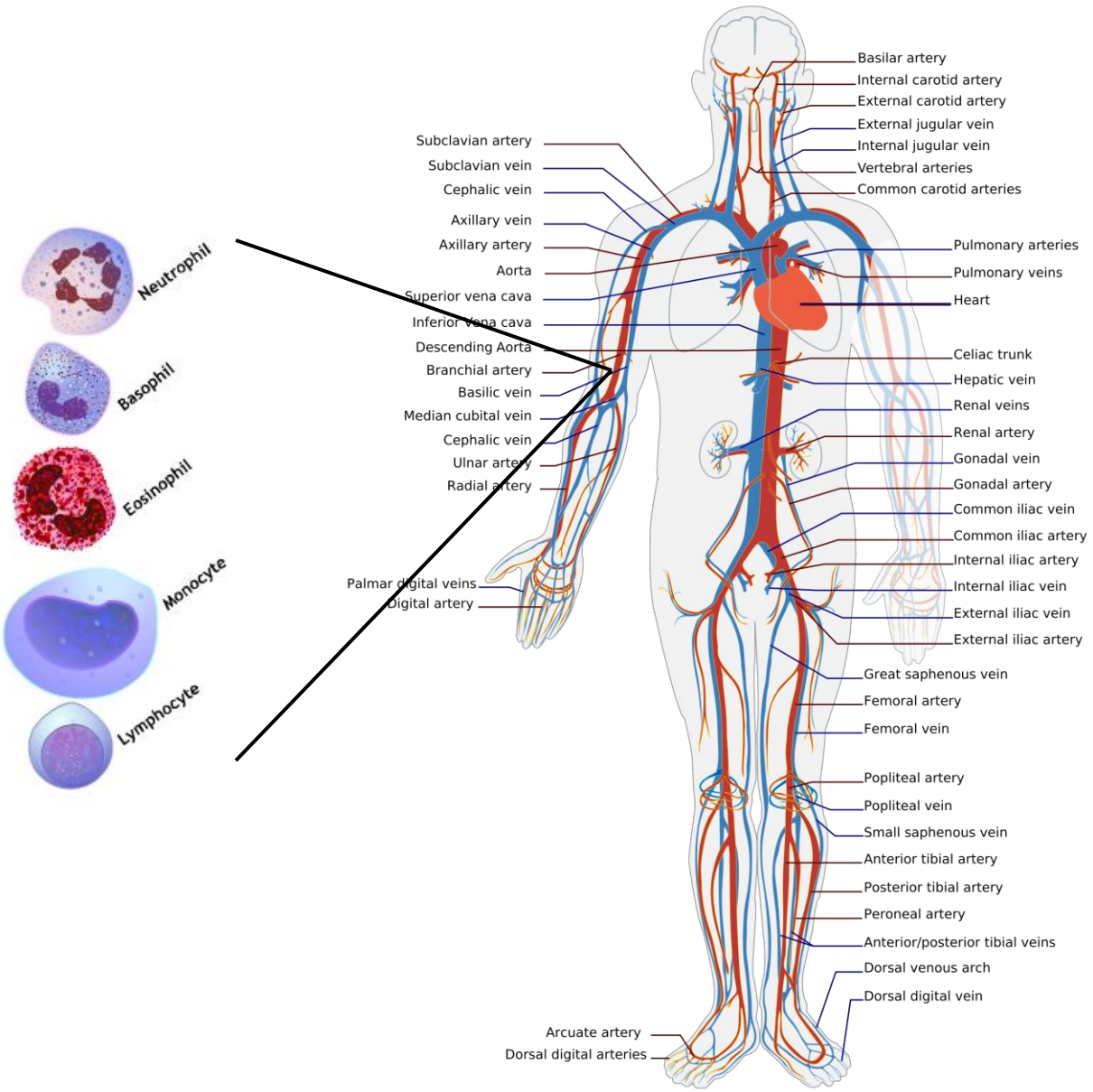
the immune system is well organized



cells circulate and we can capture them



technology allows for discovery and increases complexity

major improvements in microscopy (technological breakthrough)

flow cytometry (technological breakthrough)

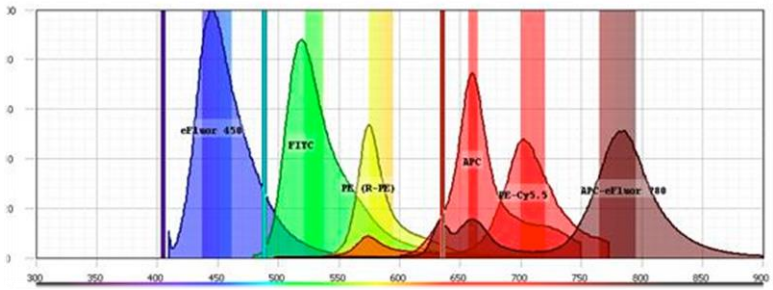
Macrophages	1880s
Mast cells, basophils & eosinophils	1890s
B cells	1950s
NK cells, T cells and DCs	1970s
Helper (CD4), cytotoxic (CD8)	1980s
pDCs vs mDCs	1990s
Th1, Th2, Th3, Th9, Th17	2000-2013
?	

Cells subsets

(A) different stages of differentiation (e.g. naïve – $CD45RA^{+}$, memory – $CD45^{-}$)

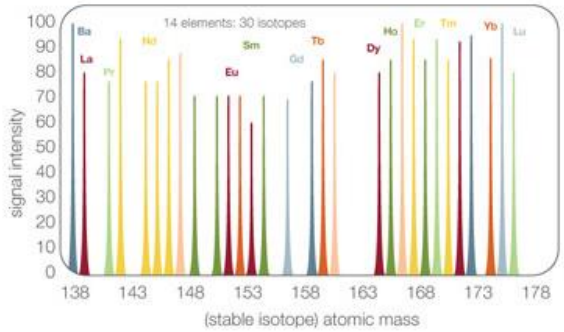
(B) different activation/functional status (e.g. quiescent – $CD69^{-}$, activated – $CD69^{+}$)

spectral overlap, an issue that has been resolved



MAX ~12 parameters

fluorophore-based flow cytometry

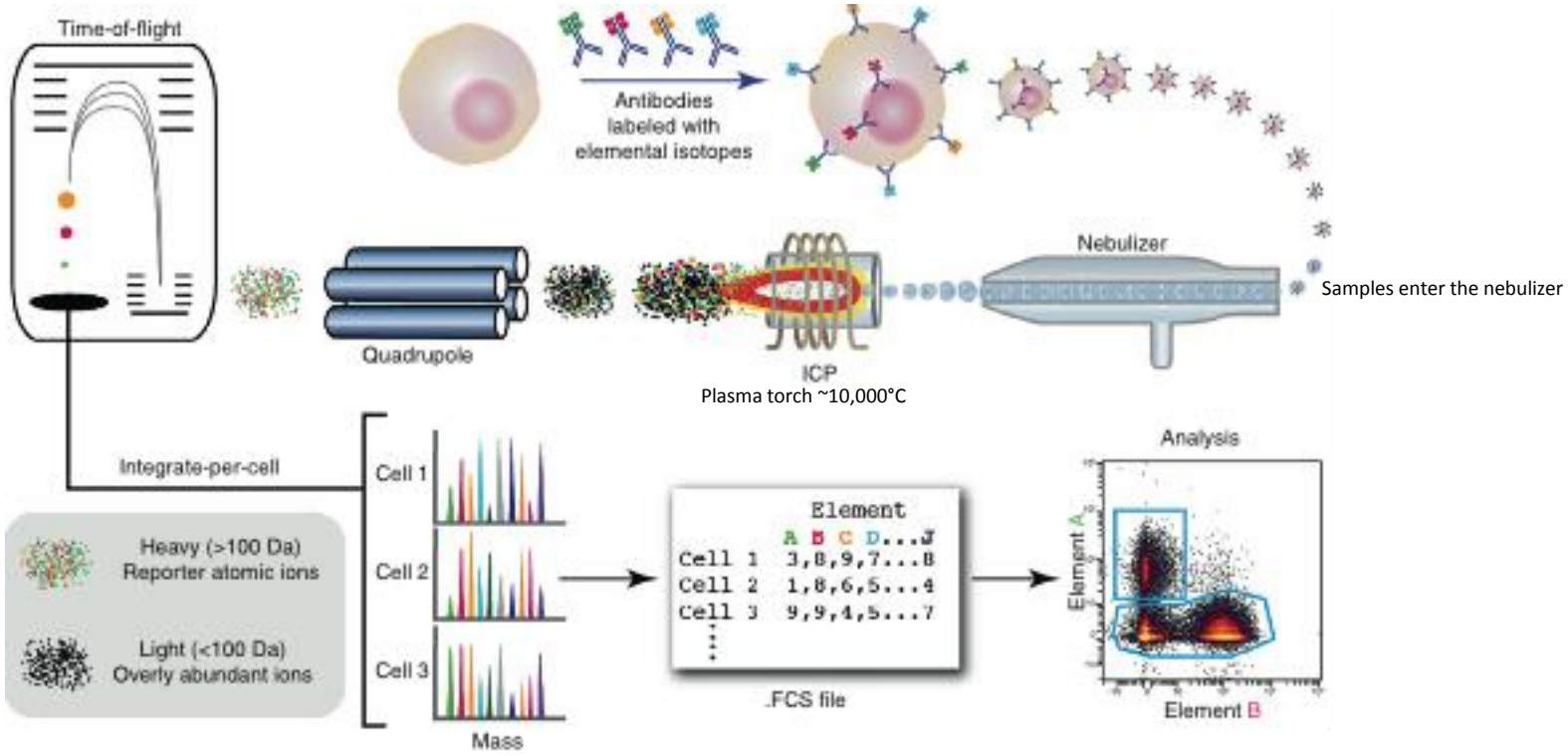


So far 40 parameters

isotope-based mass cytometry

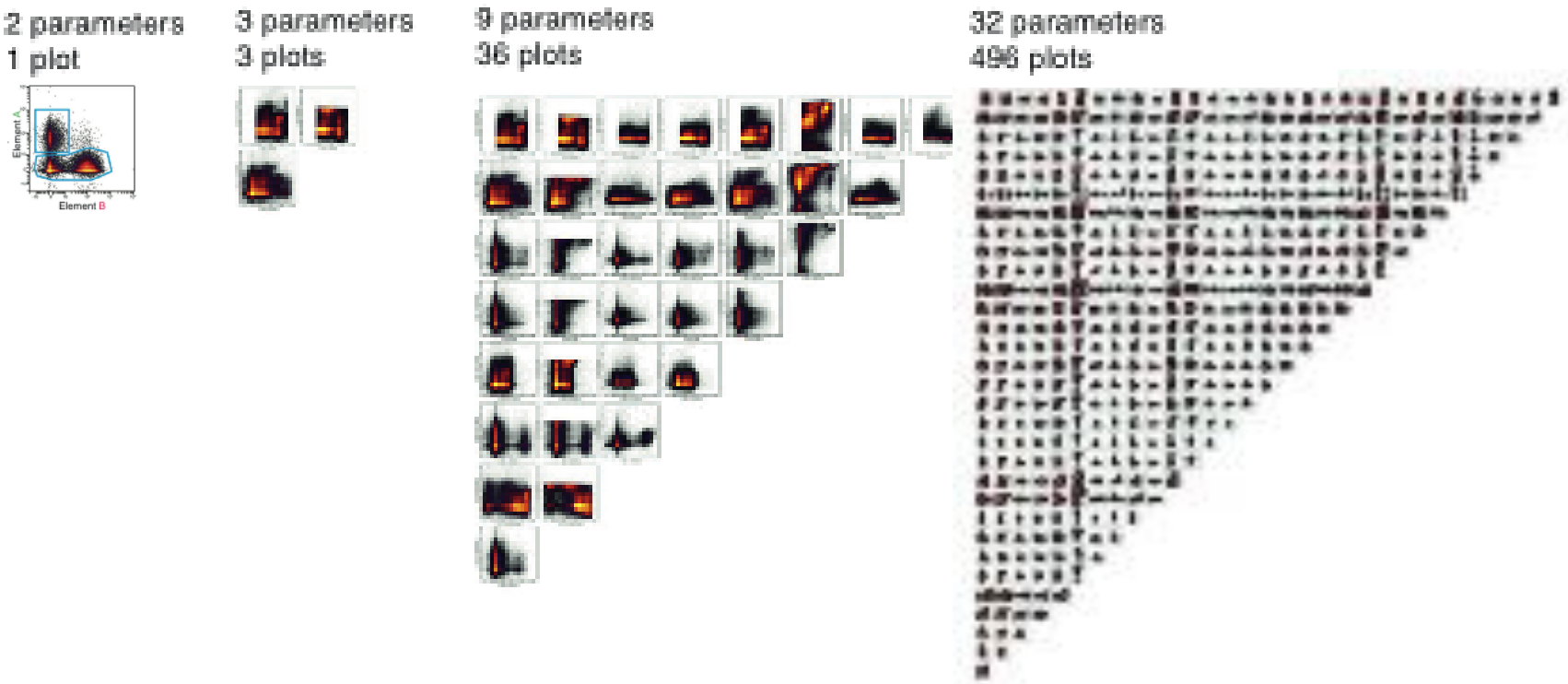
CyTOF

Inductively coupled plasma mass spectrometry (ICP-MS) (late 90s)
-> elemental composition of materials
-> ultra-trace (10^{-15} g/ml) detection of elements in environmental and clinical samples



Bendall S, et al. Science, 2011

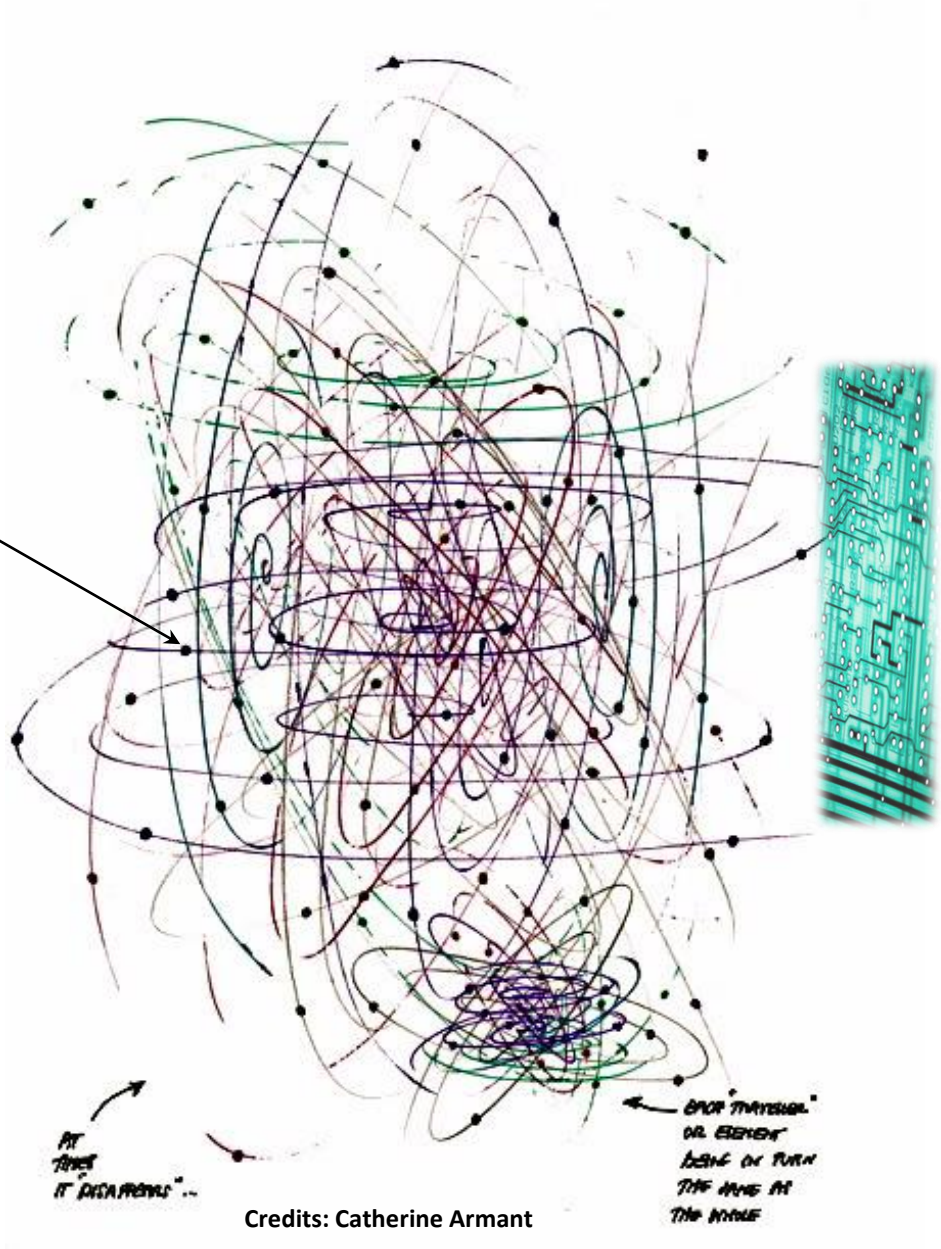
again, technology allows for discovery and increases complexity



research is changing

hypothesis-driven
(deductive)

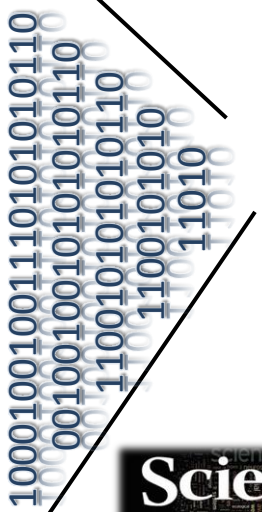
I have a hypothesis and will delete this protein from the system

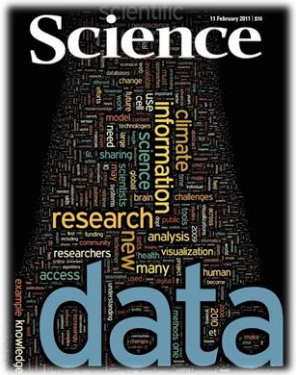


Credits: Catherine Armant

data-driven
(inductive)

let the data 'speak'

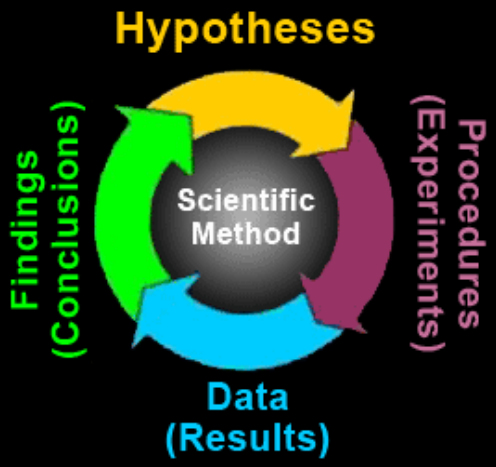




the era of data

hypothesis and data-driven research can co-exist

…and interact synergistically!!



human diversity : curse or blessing?

inbred genetics

+

controlled environment

phenotypes with small variation

mixed genetics

+

uncontrolled environment

phenotypes with large variation

systems biology : the knowing nothing (discovery) approach

1. Apoptosis is a Biomarker of Vaccine Responsiveness in Humans and Mice

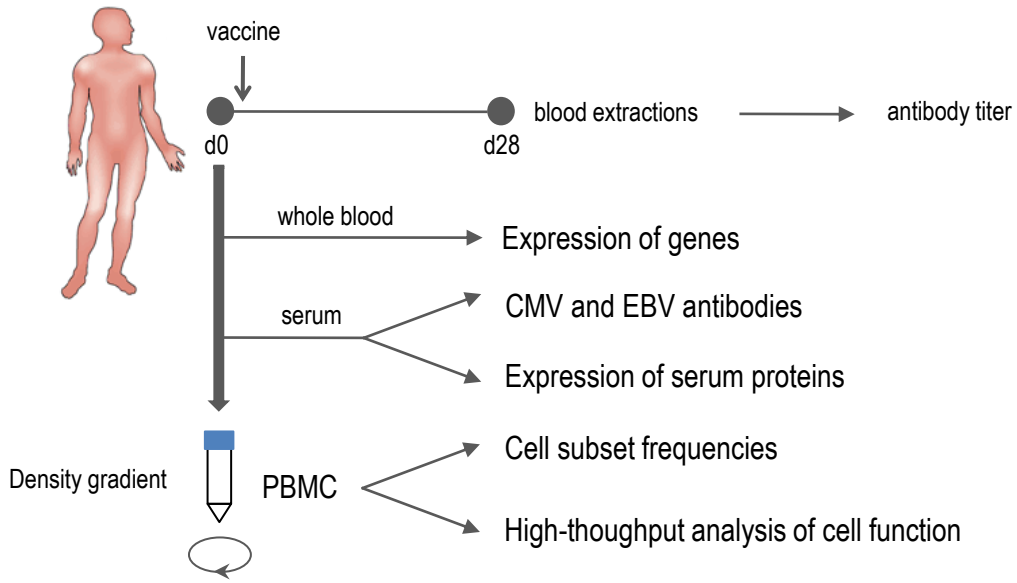
2. Cytomegalovirus Potentiates Immune Responses in Young but not Older Adults

3. Testosterone mediates weaker vaccine responses in men

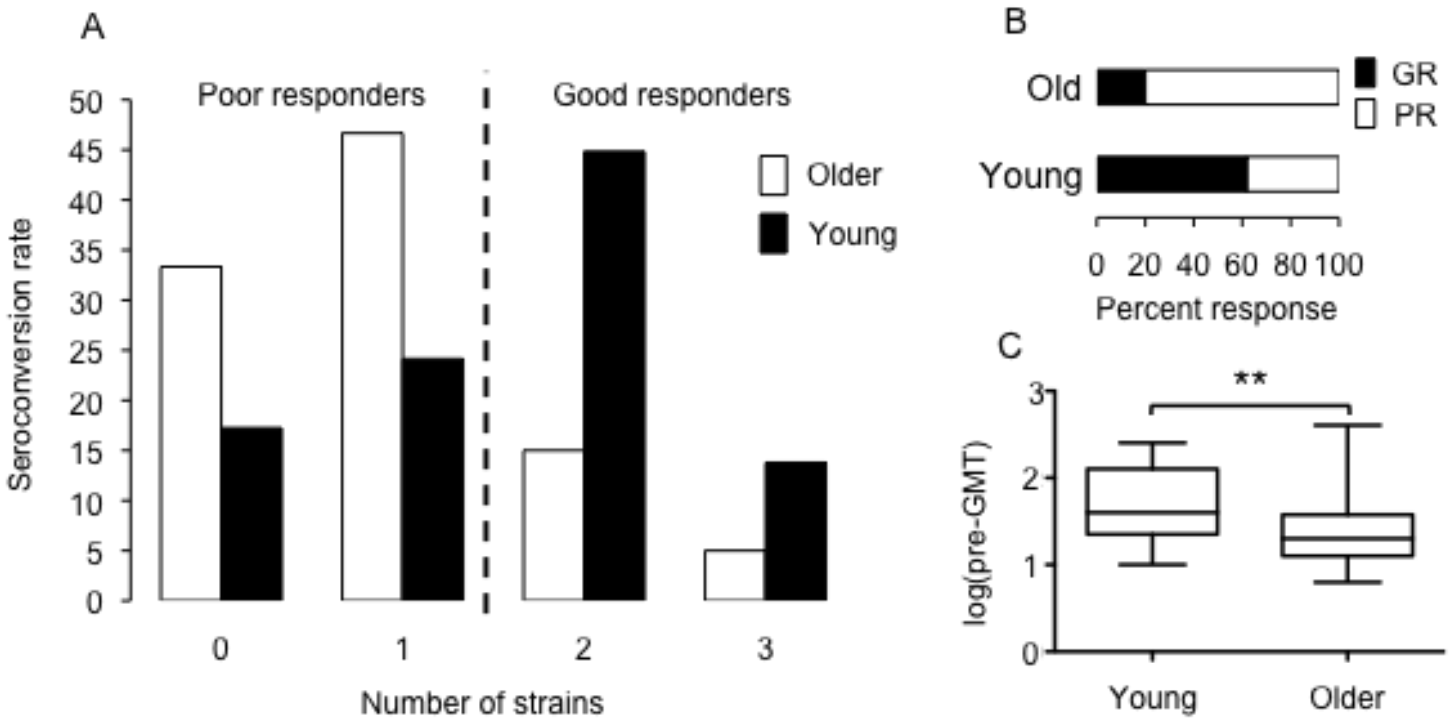
systems biology : the knowing nothing (discovery) approach

Q1: can we use blood to find mechanisms of response to immunizations?

Cohort characteristics (N= 90)
young, older, cmv-, cmv+, females and males



aging affects response to vaccination



demographics

chronic infection status

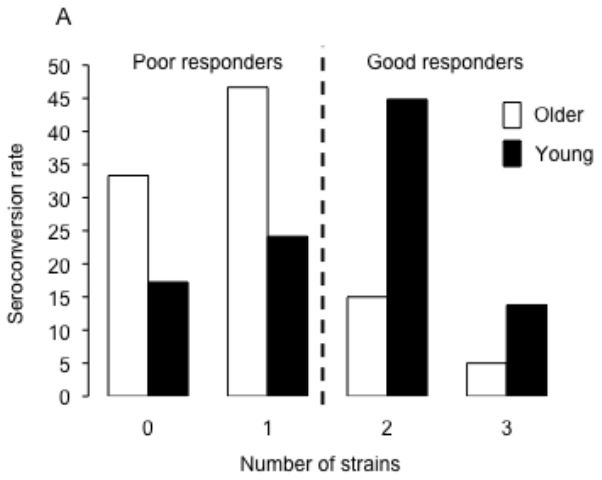
gene expression

cytokine levels

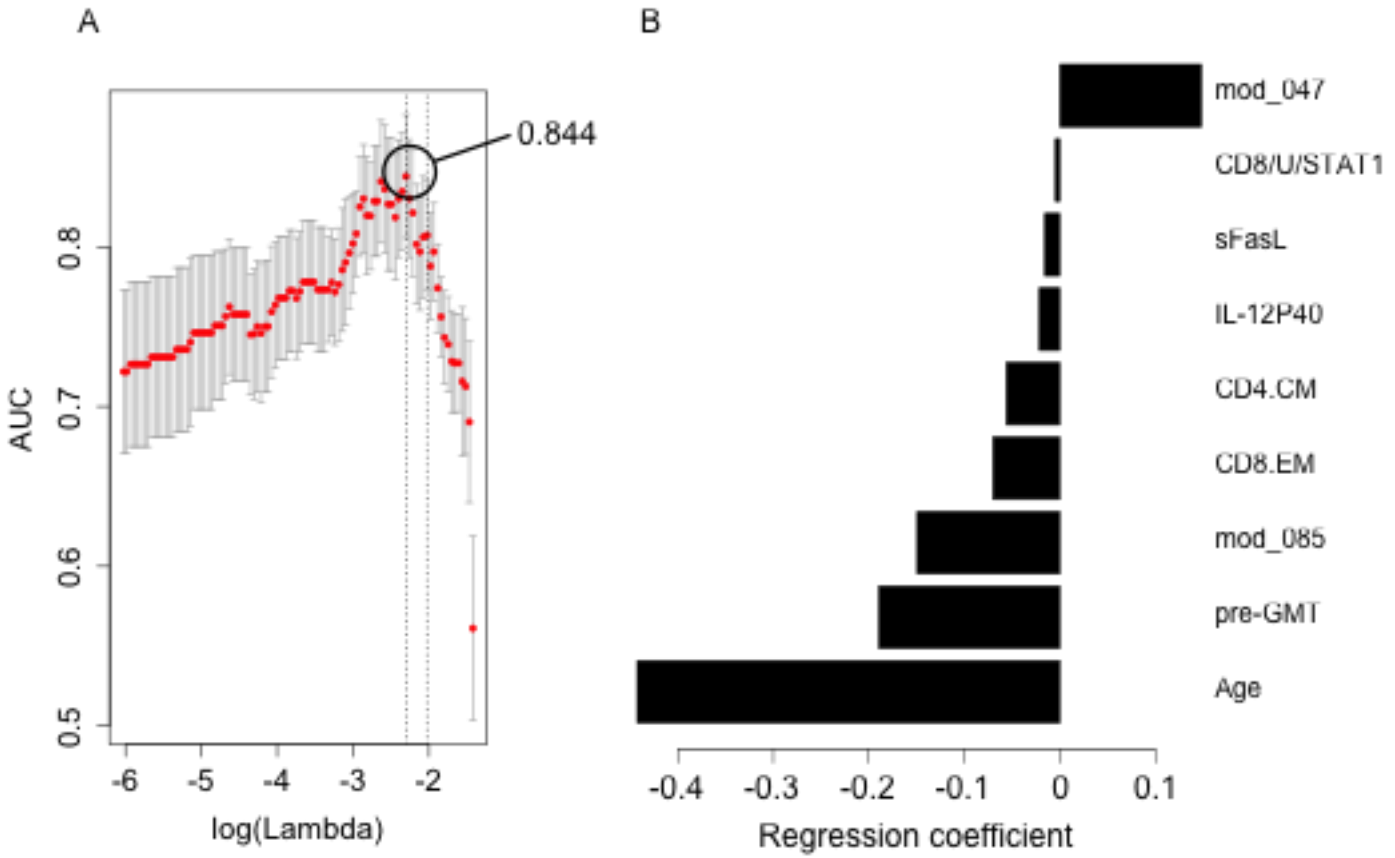
cell subset frequency

signaling responses

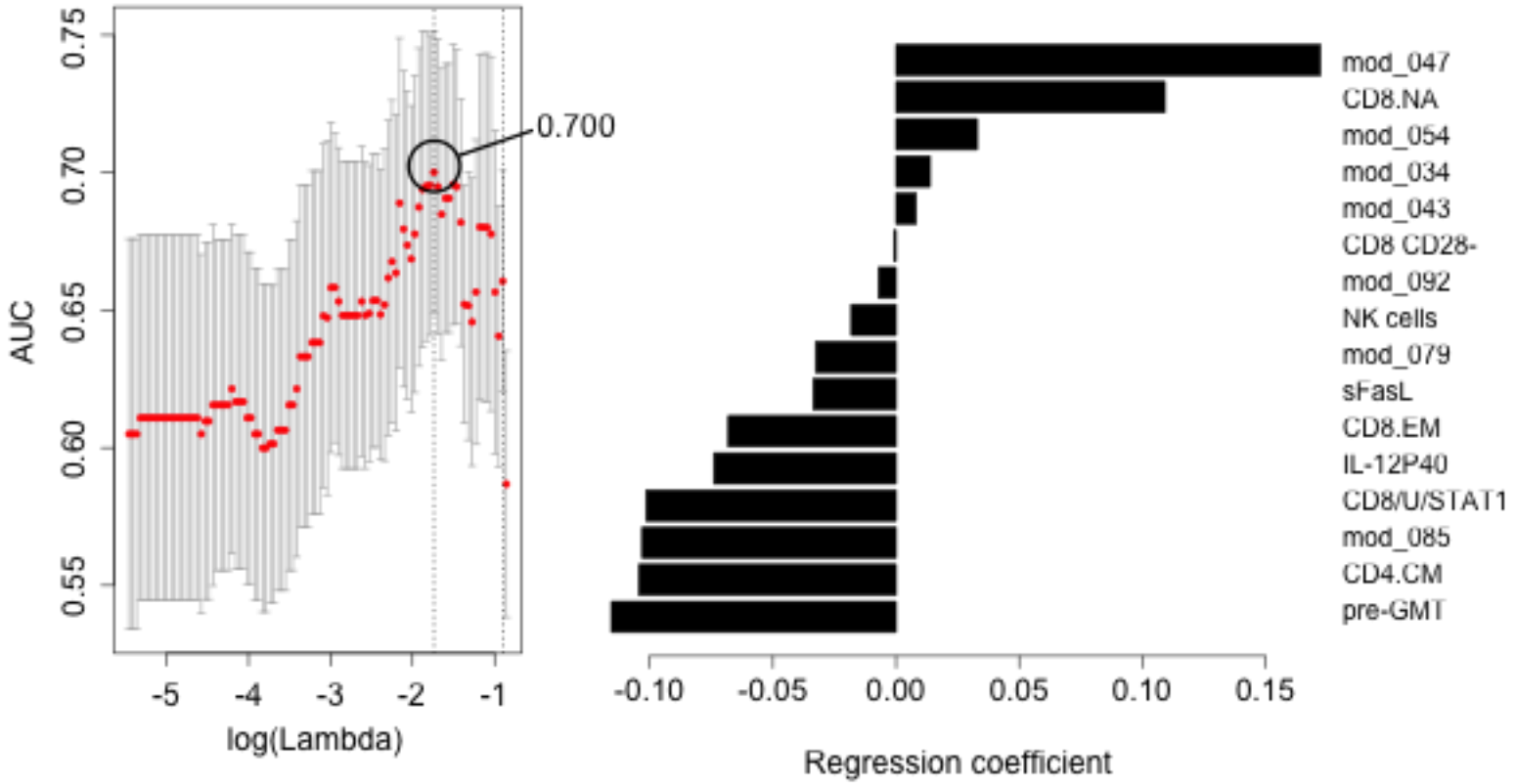
LOOCV



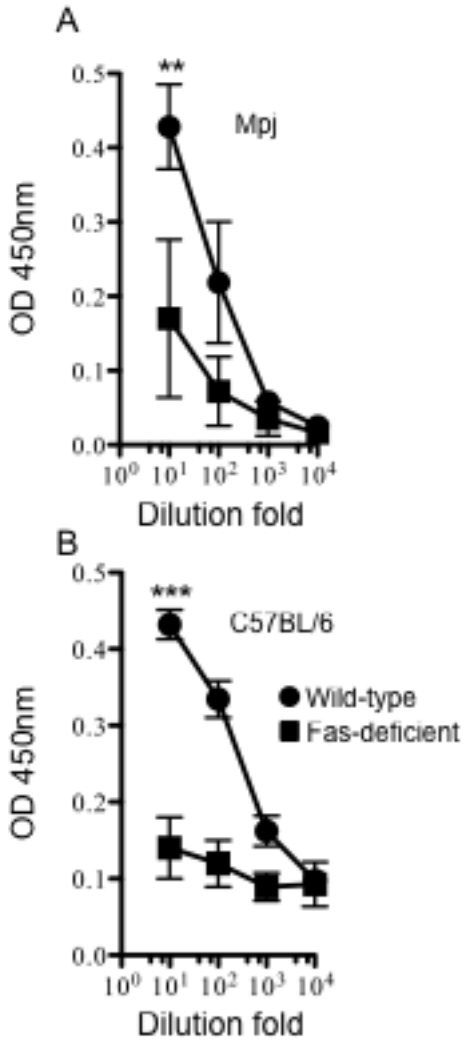
A1: yes, and apoptosis is involved



more features are discovered after removing age

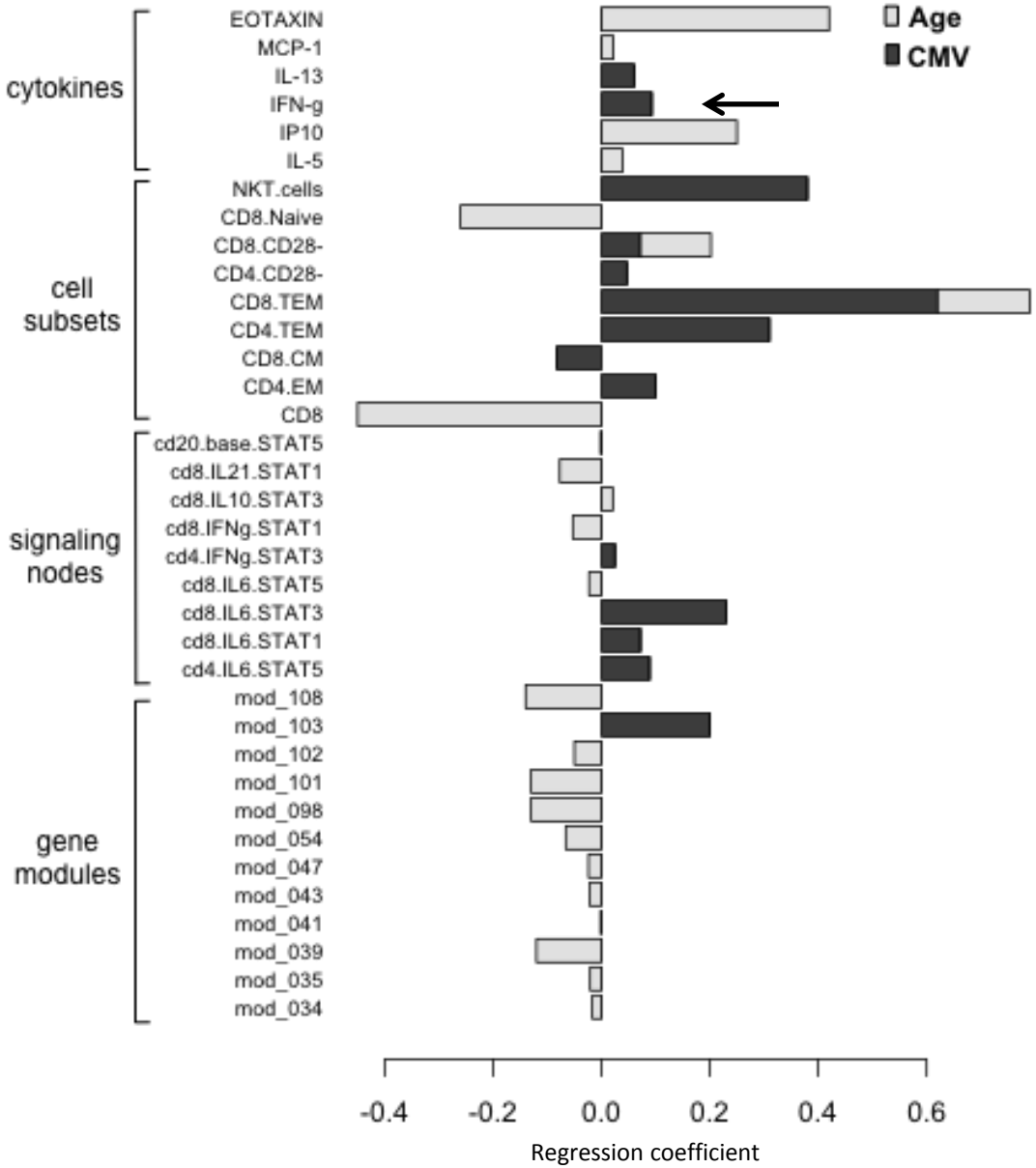


apoptosis-deficient mice have weaker responses to vaccination

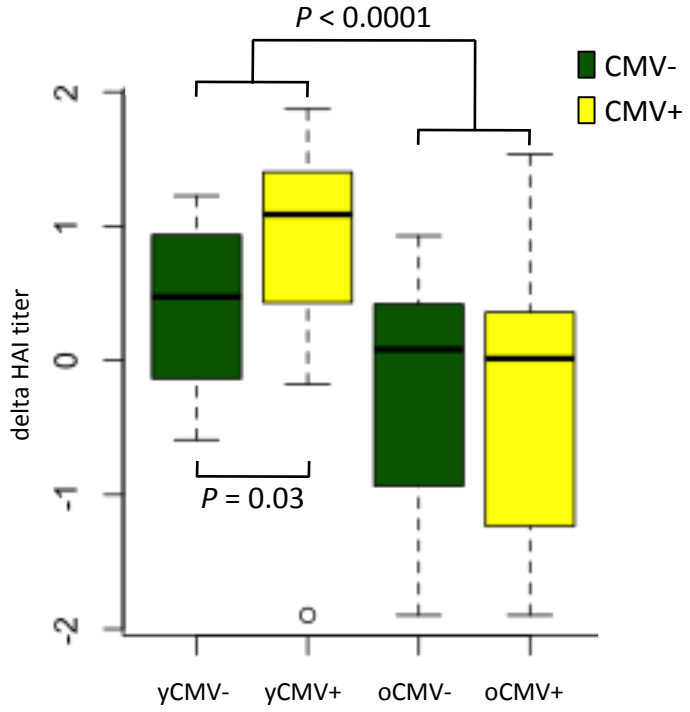


Furman D., et al. (2013) *Mol Syst Bio* 9:659

different immunological profiles in CMV infection and aging



CMV improves vaccine response in young subjects

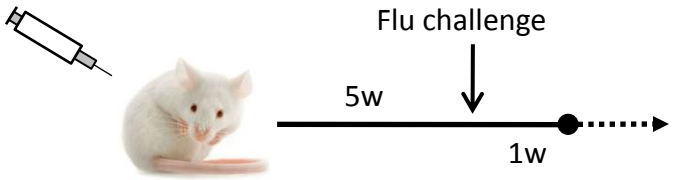


infection with mCMV prior to influenza challenge

- Influenza-disease
  - Weight loss
  - Survival
  - Flu viral titer
- CD8+ Flu-specific T cells in the BAL

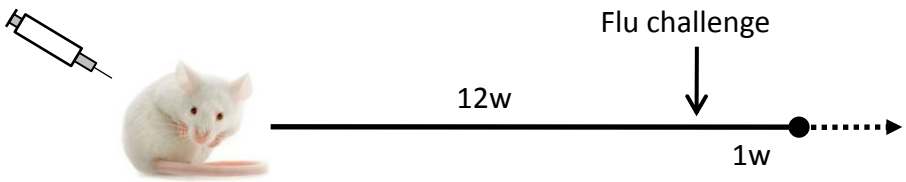
cmv

A. Early latency



cmv

B. Established latency



With Paul Thomas
St Jude Children's Hospital

cmv

C. Long-standing latency

Figure 3

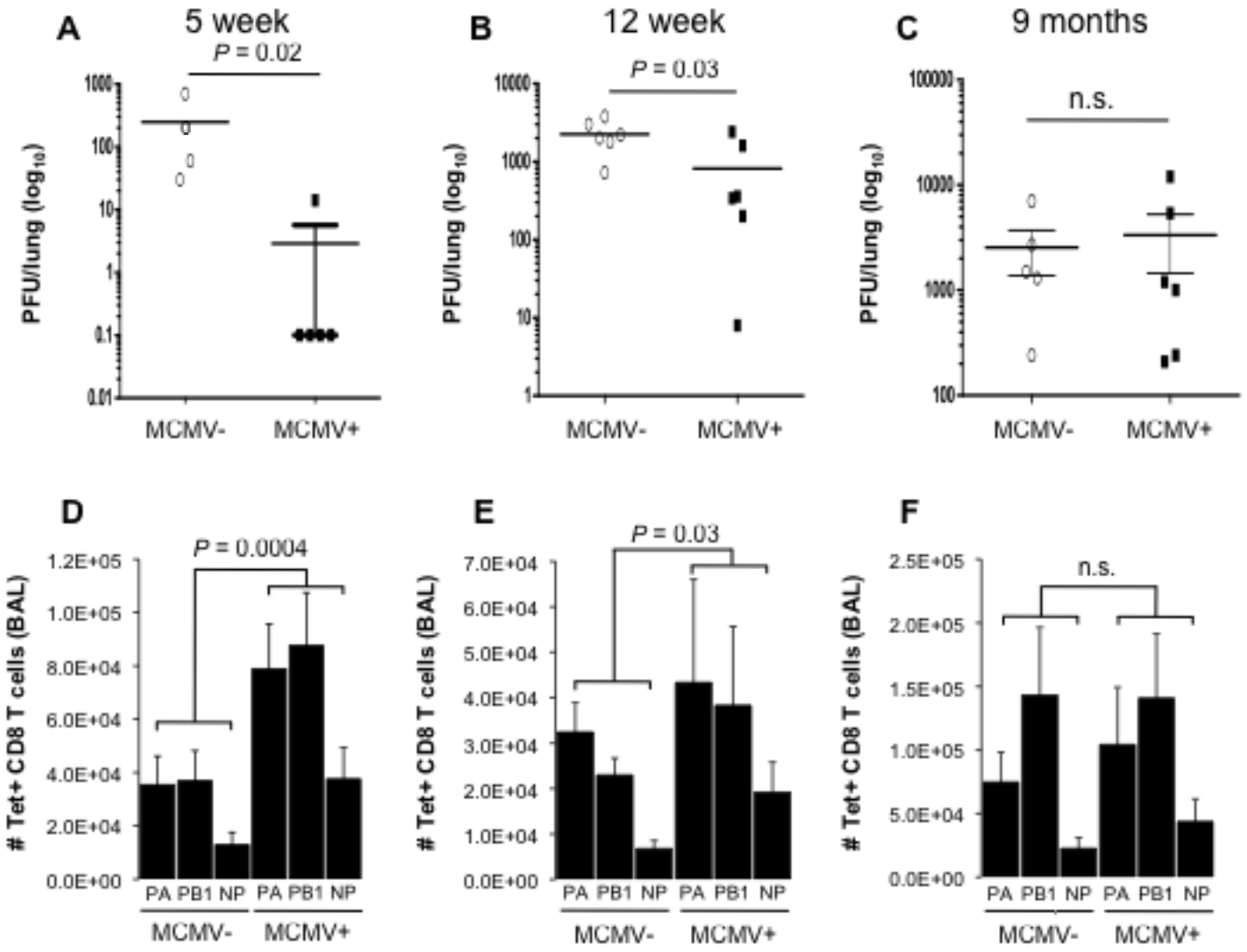
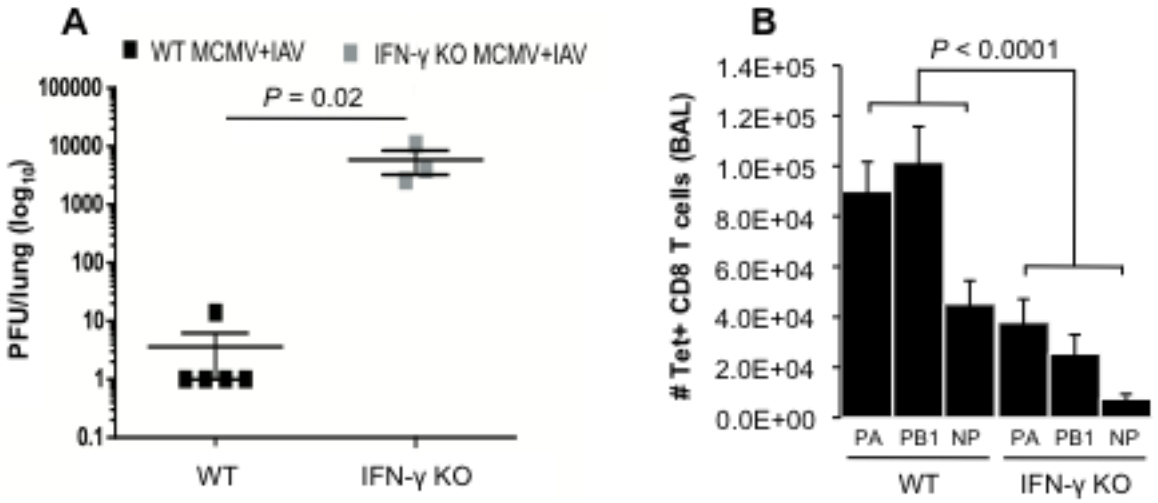

A
5 week
P = 0.02
PFU/lung (log10)
1000
100
10
1
0.1
0.01
MCMV-
MCMV+
B
12 week
P = 0.03
PFU/lung (log10)
10000
1000
100
10
1
MCMV-
MCMV+
C
9 months
n.s.
PFU/lung (log10)
100000
10000
1000
100
MCMV-
MCMV+
D
P = 0.0004
# Tet+ CD8 T cells (BAL)
1.2E+05
1.0E+05
8.0E+04
6.0E+04
4.0E+04
2.0E+04
0.0E+00
PA PB1 NP PA PB1 NP
MCMV-
MCMV+
E
P = 0.03
# Tet+ CD8 T cells (BAL)
7.0E+04
6.0E+04
5.0E+04
4.0E+04
3.0E+04
2.0E+04
1.0E+04
0.0E+00
PA PB1 NP PA PB1 NP
MCMV-
MCMV+
F
n.s.
Tet+ CD8 T cells (BAL)
2.5E+05
2.0E+05
1.5E+05
1.0E+05
5.0E+04
0.0E+00
PA PB1 NP PA PB1 NP
MCMV-
MCMV+

Figure 4



Q3: what mediates the improved response in women?

Sexual dimorphism in the human immune system

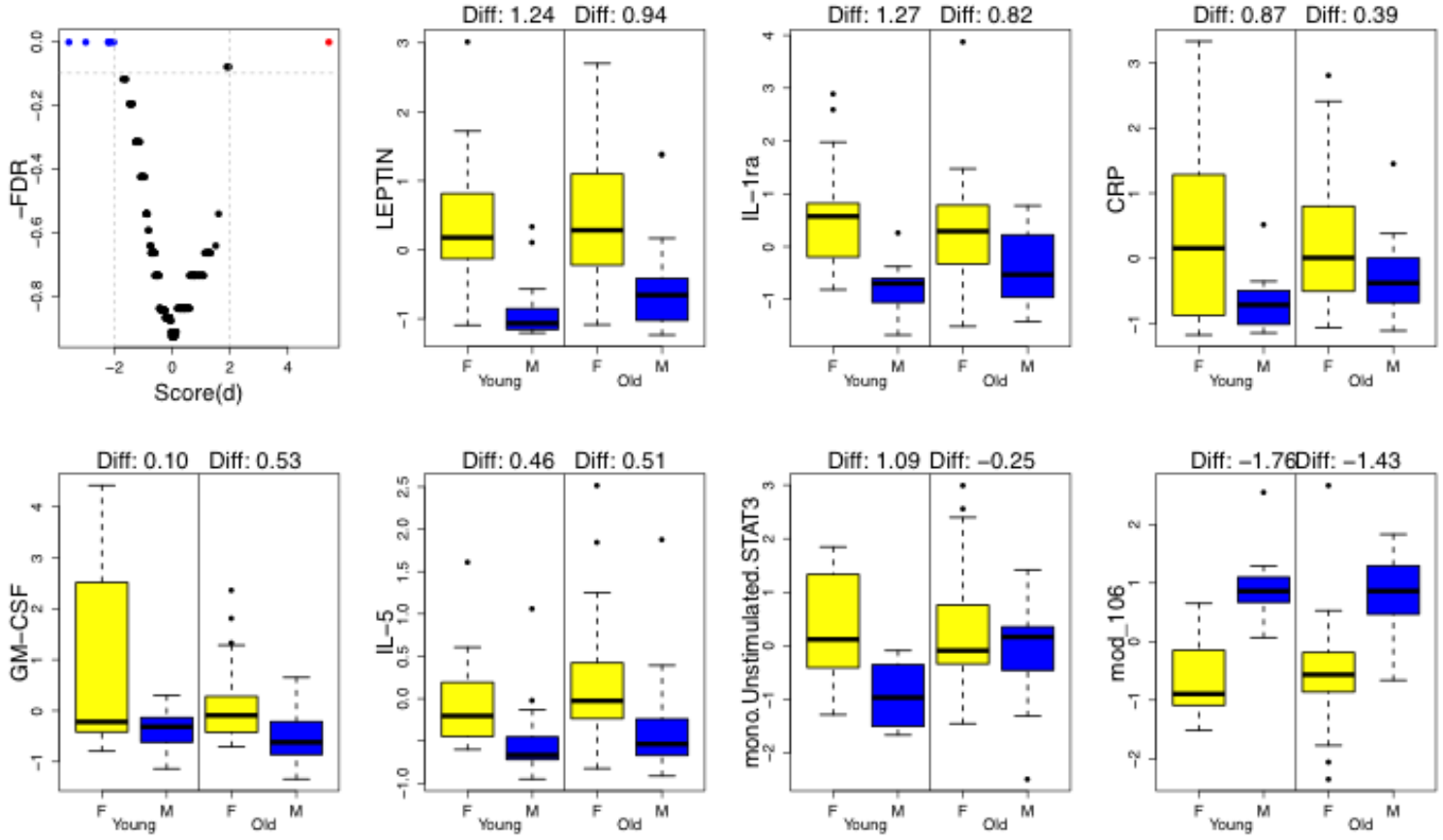
1. longer lifespan

2. stronger response to infection & vaccination

3. more resilient to cancer

4. more susceptible to autoimmune diseases

sex differences in inflammatory markers



3: gender (sex)

dimorphism in the antibody neutralizing activity

		Beta	*Std. Error*	*z value*	*P-value*
H1N1	(Intercept)	-0.272	0.229	-1.190	0.234
	Age	-0.690	0.236	-2.919	0.004
	Gender	-0.011	0.234	-0.047	0.962
H3N2	(Intercept)	-0.038	0.228	-0.166	0.868
	Age	-0.190	0.236	-0.804	0.421
	Gender	-0.716	0.239	-2.992	0.003
B	(Intercept)	-0.502	0.236	-2.128	0.033
	Age	-0.583	0.246	-2.367	0.018
	Gender	-0.594	0.256	-2.324	0.020

is there a feature that can explain the differences?

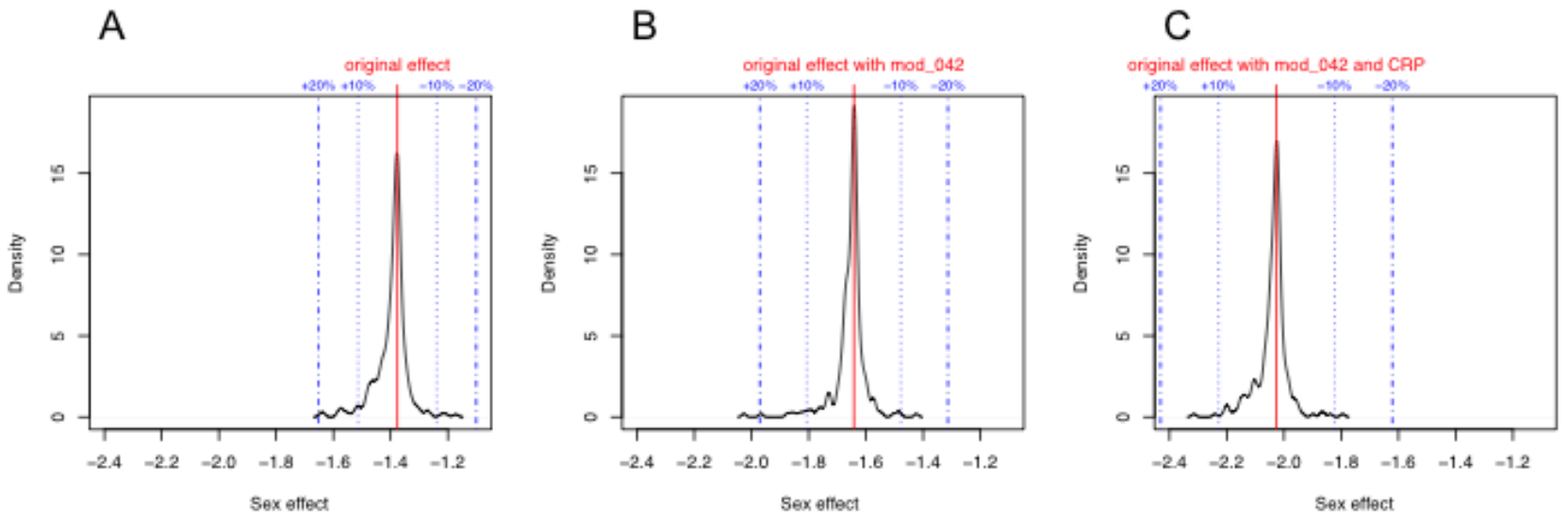
(i) The *Gender* has a direct impact on the *Vaccine response*

(ii) Some feature may be intermediate factors in the effect of *Gender* on the *Vaccine response*

search for confounding factors

forward stepwise regression

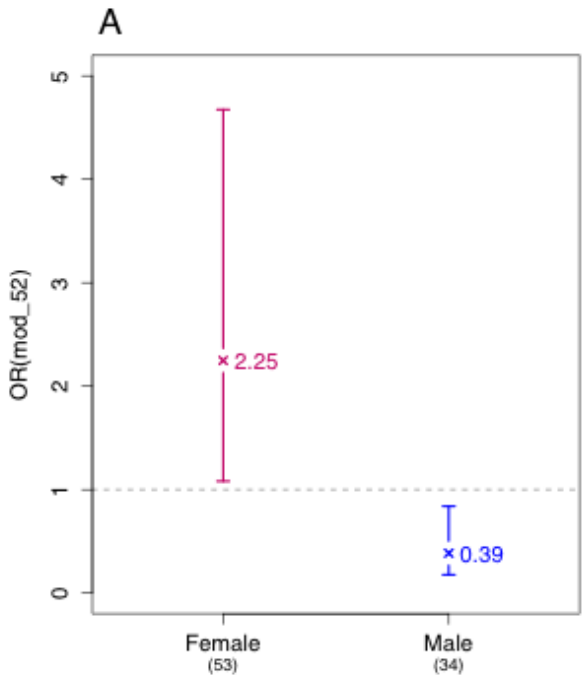
$$\text{logit}(y) \sim \text{sex}*\beta_{sex} + x*\beta_x + \varepsilon$$



search for interactions with sex that explain vax response

1. Test for significant interactions (FDR < 0.1)
2. Fit model to estimate OR

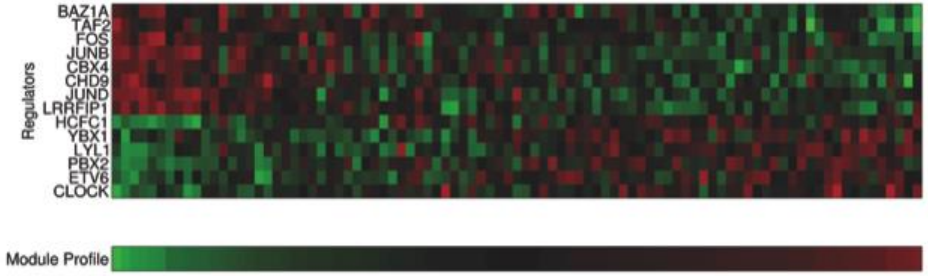
$$\text{logit}(y_i) = \mu + \beta_g \cdot male_i + \beta_c \cdot crp_i + \beta_{m42} \cdot mod\_42_i + \beta_{m52} \cdot mod\_52_i + \boxed{\beta_{g:m52} \cdot male_i : mod\_52_i} + \varepsilon_i$$



Module 52 – immunosuppression*

35 genes
Lipid biosynthesis ($P < 0.0001$)
Interesting genes: LTA4H, PDSS2

RP > BAZ1A, FOS, JUNB (repressors)



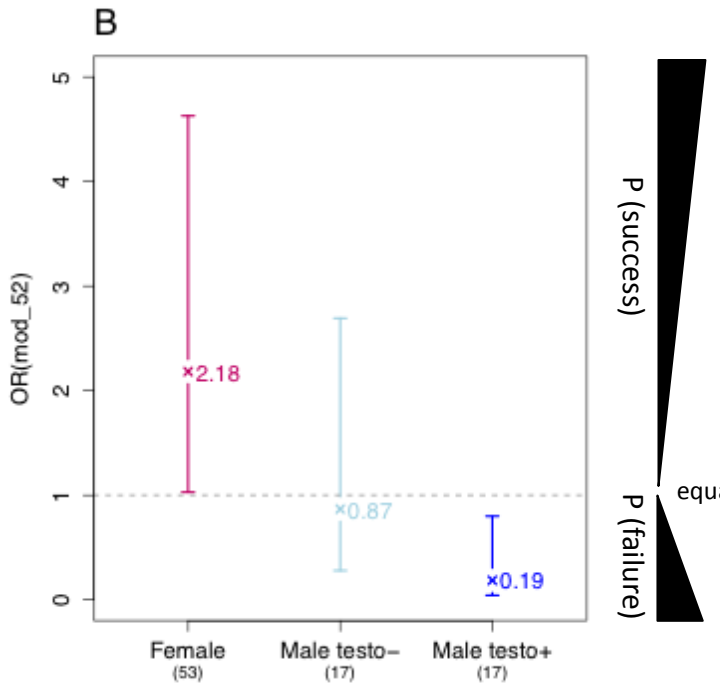
Gene chemical association for testosterone $P = 0.0003$

* Yokota Y, *et al.* (2012) *Blood* 120(17):3444-3454.
Juzan M, Hostein I, & Gualde N (1992) *Prostaglandins Leukot Essent Fatty Acids* 46(4):247-255.
Kanneganti TD & Dixit VD (2012) *Nat Immunol* 13(8):707-712.

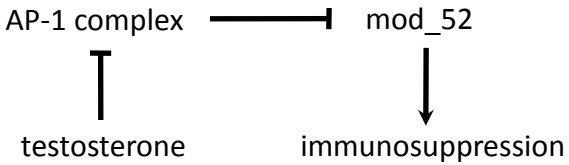
Simon, N. and Tibshirani, R. (2012) A Permutation Approach to Testing Marginal Interactions in Many Dimensions

lowest response to TIV in males with highest testosterone and expression of module 52

$$\text{logit}(y_i) = \mu + \beta_c \cdot crp_i + \beta_{m42} \cdot mod\_42_i + \beta_{m52} \cdot mod\_52_i + \beta_a \cdot age_i + \beta_{Tlo} \cdot maleTlo_i + \beta_{Tlo:m52} \cdot maleTlo_i : mod\_52_i + \beta_{Thi} \cdot maleThi_i + \beta_{Thi:m52} \cdot maleThi_i : mod\_52_i + \varepsilon_i$$



equal probability of being a good or poor responder



Furman, D. et al. *PNAS* doi 10.1073/pnas.1321060111

conclusions

- Apoptosis is important for the ability to respond to TIV
  - Making room for the generation of new memory cells?
- CMV potentiates the Ab response to TIV
  - Effect is lost with time
- Testosterone seems to modify expression of genes that participate in suppression of the Ab response to TIV

acknowledgements

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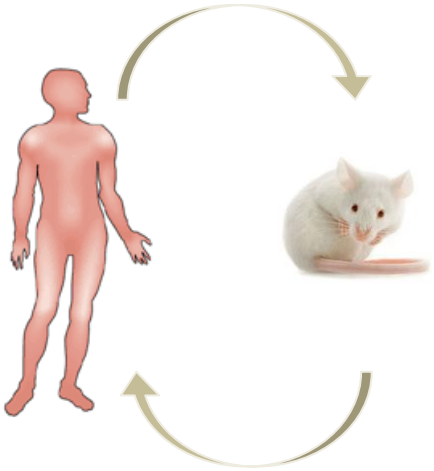

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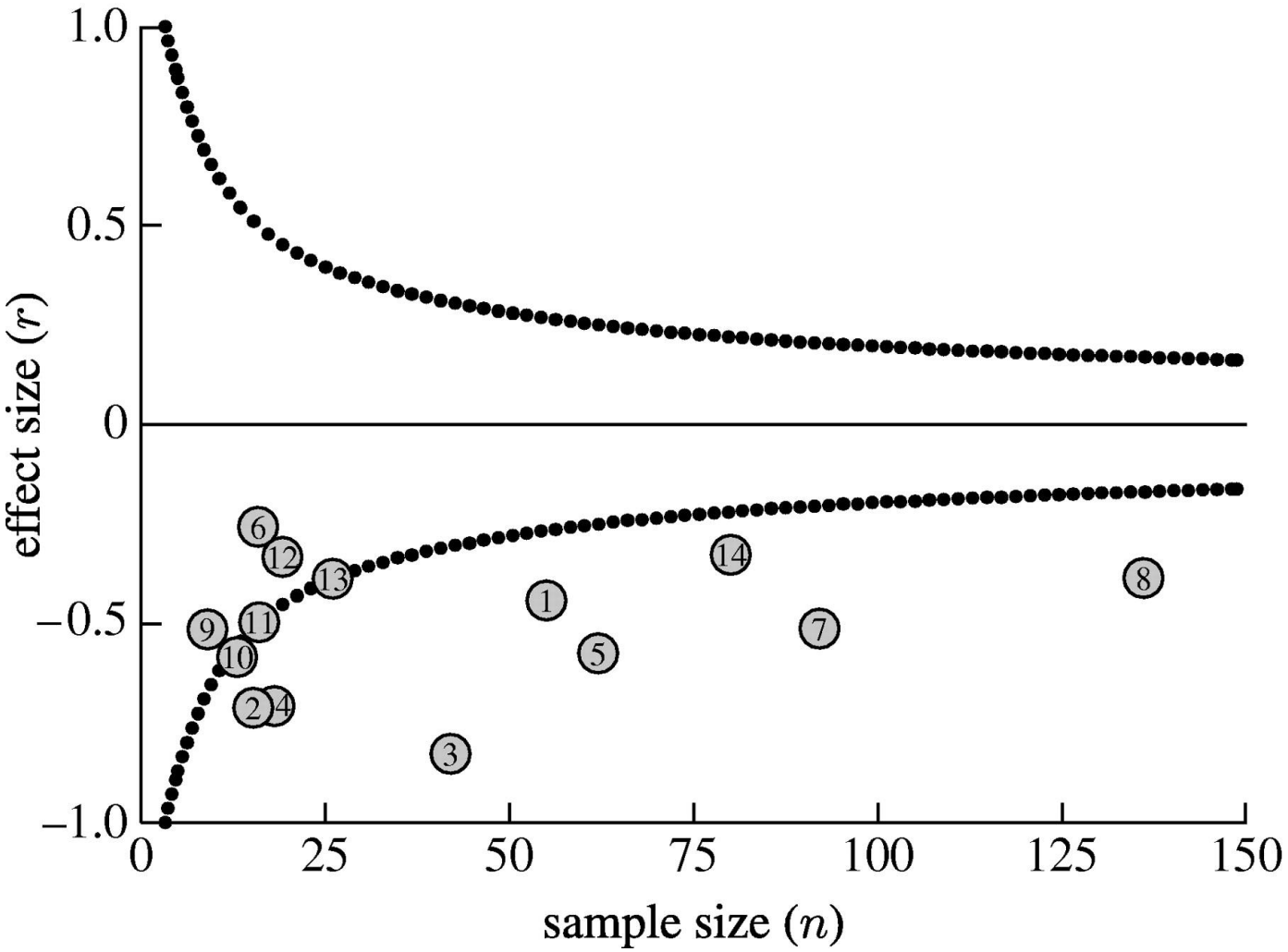
HHMI HOWARD HUGHES MEDICAL INSTITUTE

Funnel plot of effect size against sample size.



Boonekamp J J et al. Biol. Lett. 2008;4:741-744

biology letters