Effector Array: data and analysis

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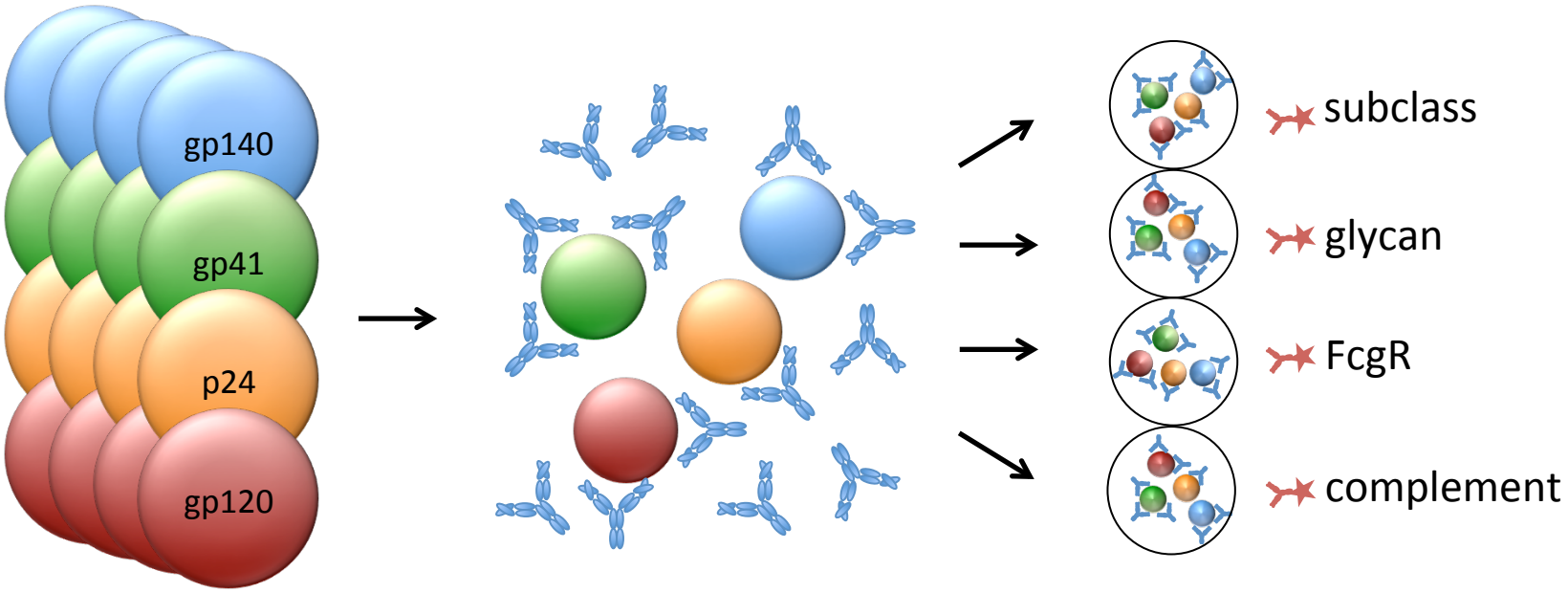
2/21/13

Luminex Bead Assay



<500 Ag-specificities

simultaneously

~20 Fc features

separately

Current Status (Antigens)

HIV gp120s	Other Env	Other HIV	Flu Antigens	Other Viral	Controls
gp120 YU2 (37)	**gp41 (HXBc2) (27)**	**p24 (HXBc2) (26)**	Flu MNA (13)	HAV P2C-P3A (4)	ahuIgG (34)
gp120 Chiang Mai (14)	**gp140 (HXBc2) (52)**	p24 IIIb (33)	Flu N2 (15)	HSV-1 gG (5)	ahuIGM (12)
gp120 from Ecoli(54)	gp140 CN54 (39)	HIV1 Integrase (28)	Flu BNA (18)	HCV E2 (6)	ahuIgA (51)
gp120 93TH975 (19)	gp140 Du151 (75)	HIV1 Nef (29)	HA Brisbane/10/07 (44)	PV1 (polio vaccine)	tetanus Ag (54)
gp120 BAL (65)	SOSIP (45)	HIV1 Rev (wt) (36)	HA NewCal/20/99 (42)	PV2	
gp120 IIIB (CHO) (25)		Vif (Ecoli) (38)	HA Wisconsin/67/05 (43)	PV3	
gp120 PVO (35)		HIV pr55 Gag (20)	HA (ΔTM) Perth/16/09 (1)		
gp120 CM235 (77)			HA (ΔTM) Florida/06 (2)		
gp120 CM244 (78)			HA1 SolomonIsl/06 (3)		
gp120 JRCSF (63)					
gp120 MN (72)					
gp120 Du151 (73)					
gp120 SF162 (74)					
gp120 ZM109F (76)					
gp120 TRO (61)					
gp120 Du156.12 (62)					
gp120 RSC3 (55)					

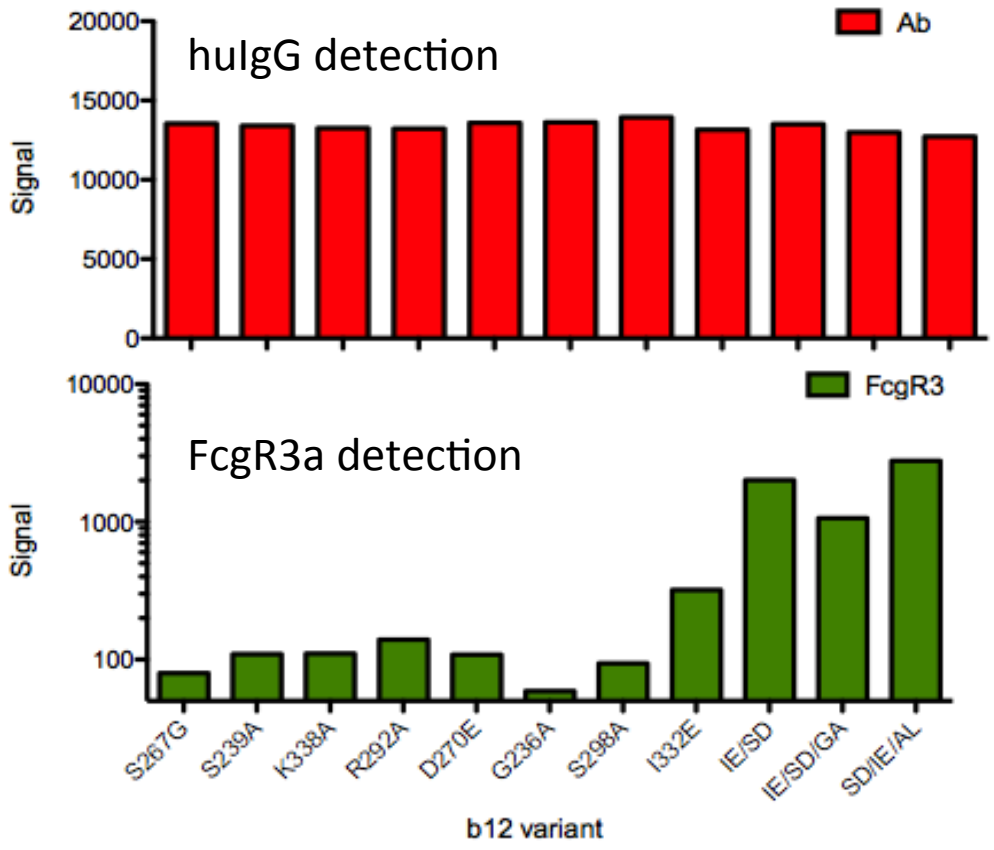
~50 Ag; currently working on whole HIV virus bead sets

Current Status (detection)

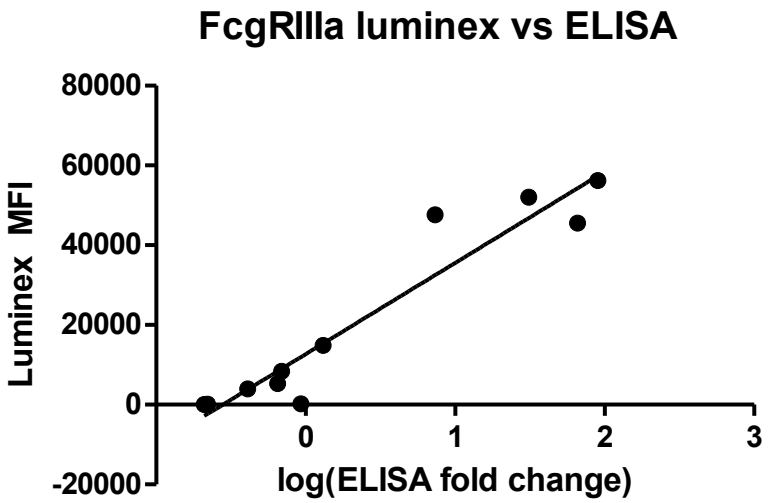
Subclassing Reagents	FcgRs	Complement Proteins	Lectins
anti-IgG1	FcgRIIa	C1q	SNA
anti-IgG2	FcgRIIb	MBL	WGA
anti-IgG3	FcgRIIIa	C3b	ConA
anti-IgG4	FcgRIIIb	C4b	GNL
	FcgRI		MAL1
	FcRn		LCA
			RCA
			PNL
			AAL
			VVL
			UAE1

Key
Works on all bead sets
Works on some bead sets
Some issues
Currently not working

Validating FcgR Data

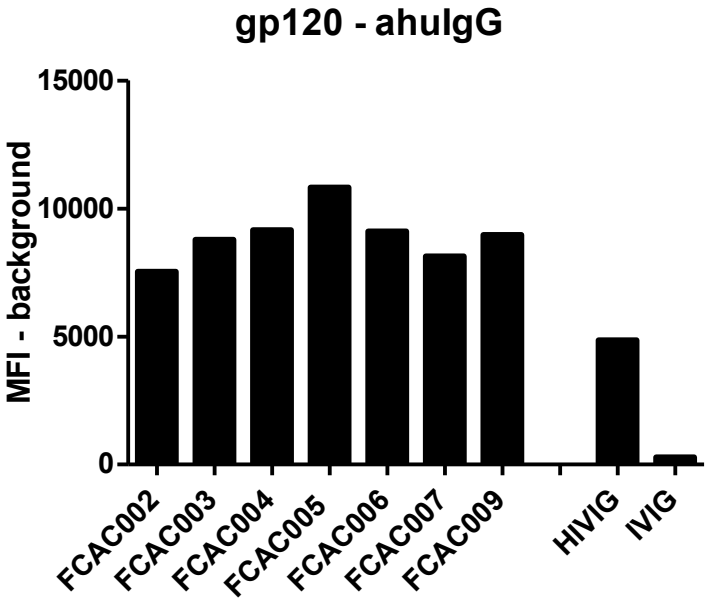


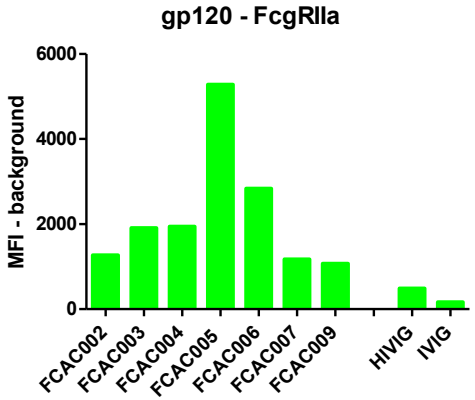
- Fc-mutant B12 antibodies from Brian Moldt (Burton Lab - Scripps).
- Compare published ELISA binding to Luminex MFI.

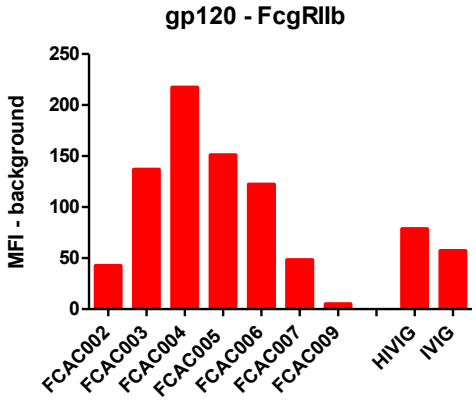


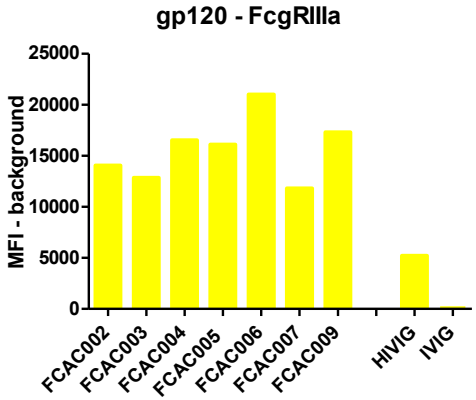
Brown et al, in preparation

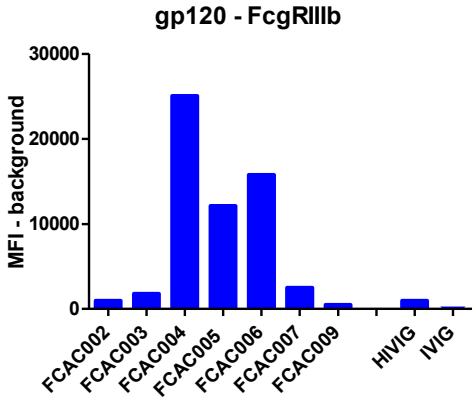
Clinical samples exhibit differential FcgR binding





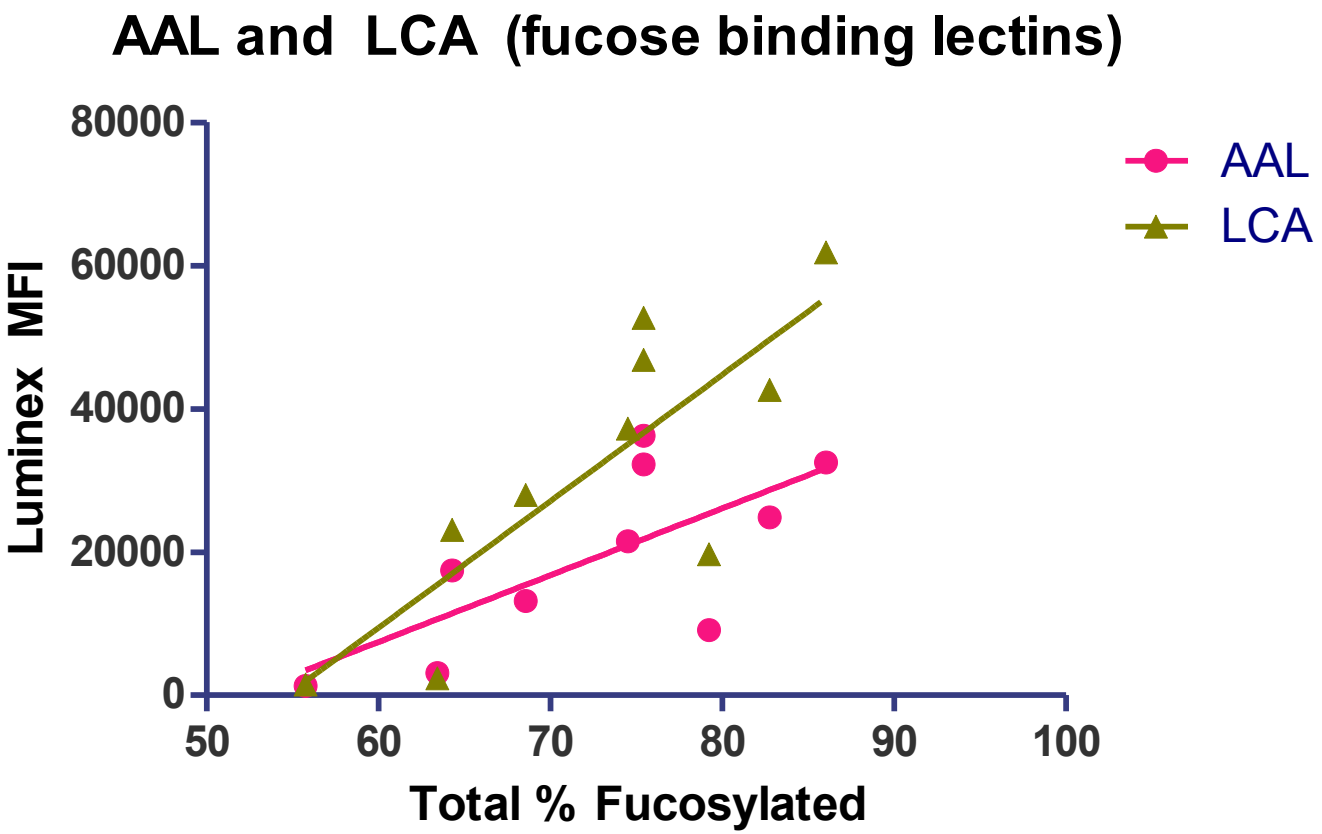






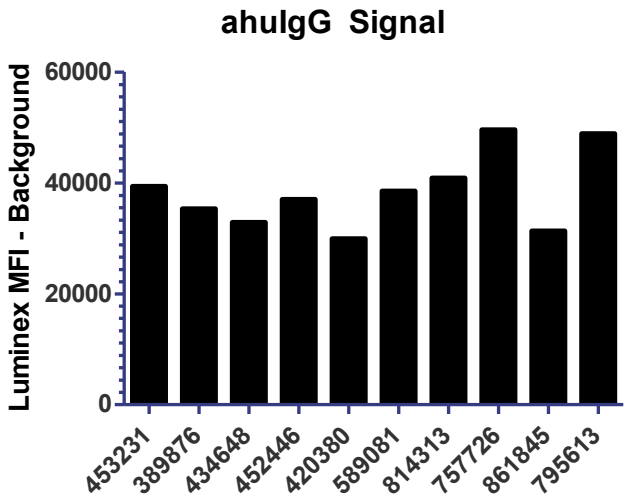
Brown et al, in preparation

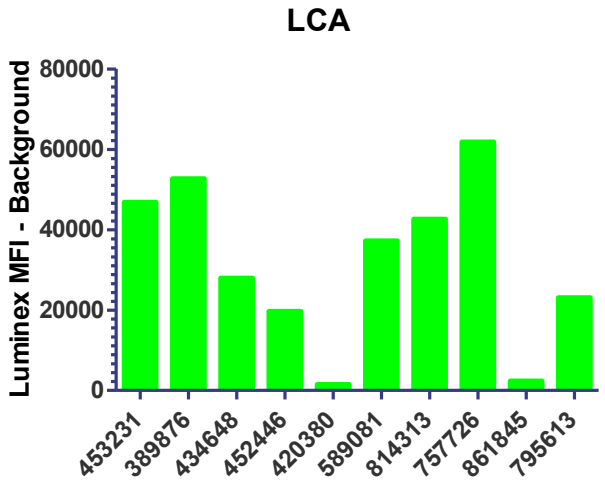
Validating lectin binding

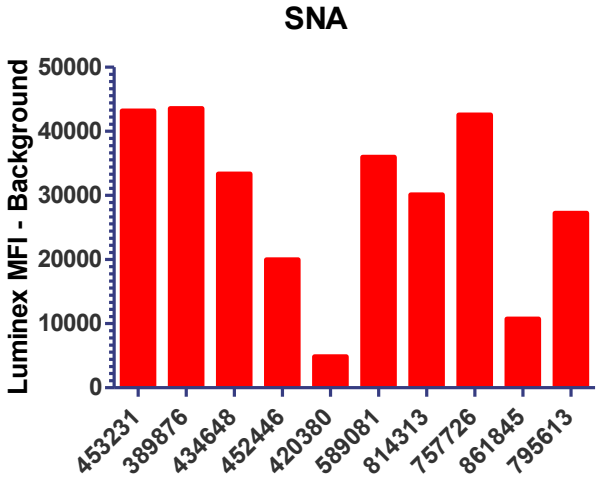


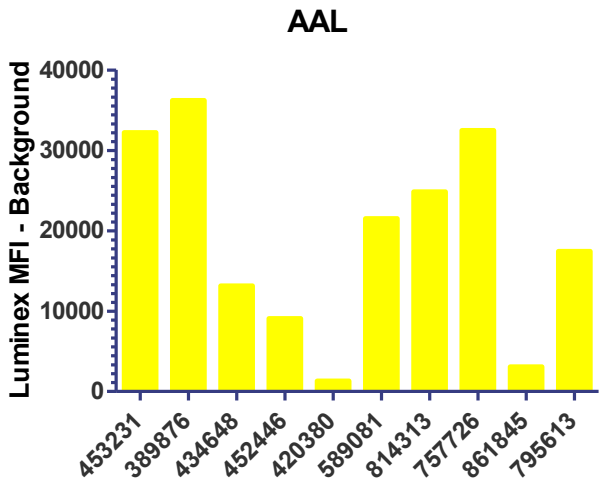
Lectin signals on array correlate with HPLC glycan data

Lectin binding differs among subjects

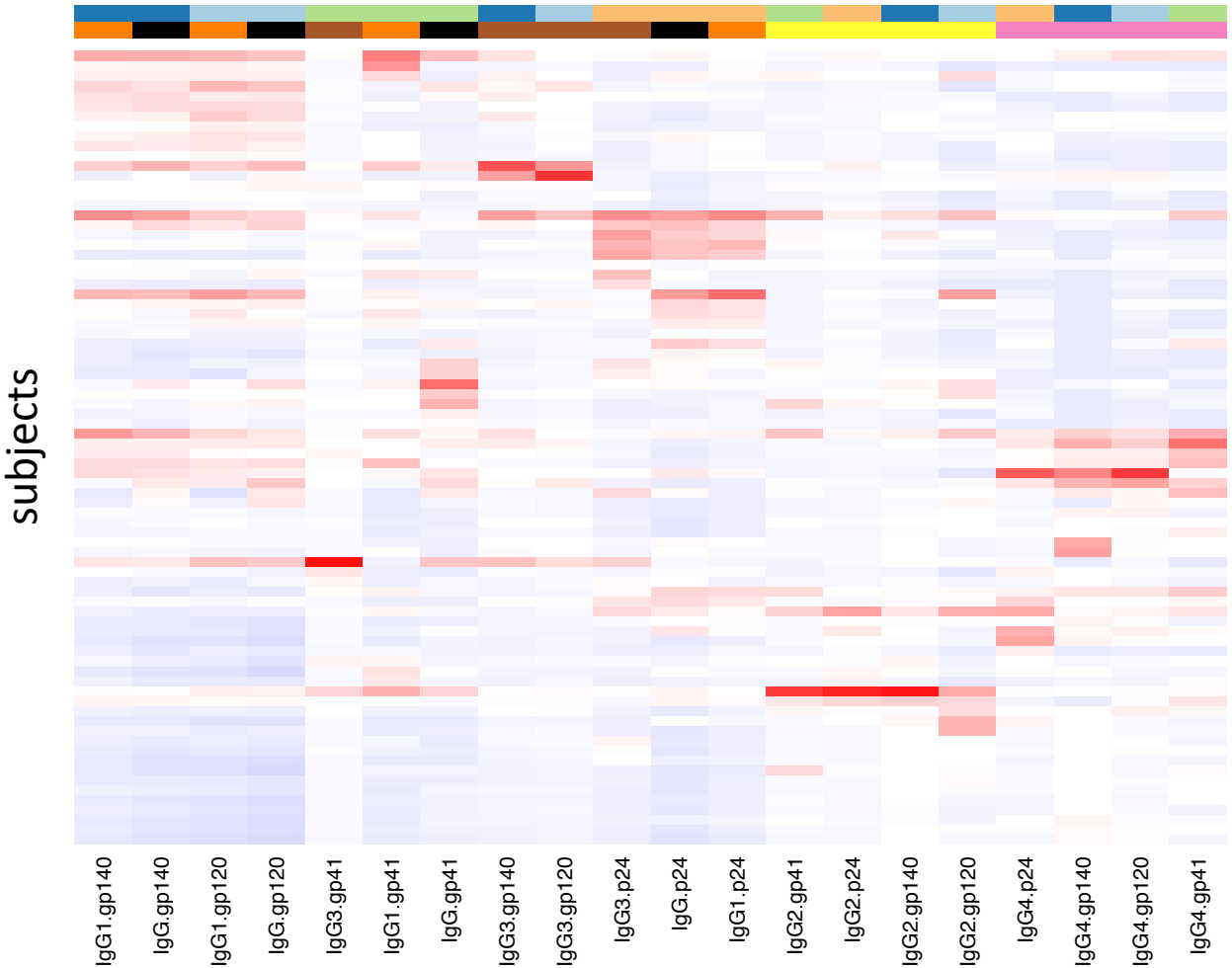


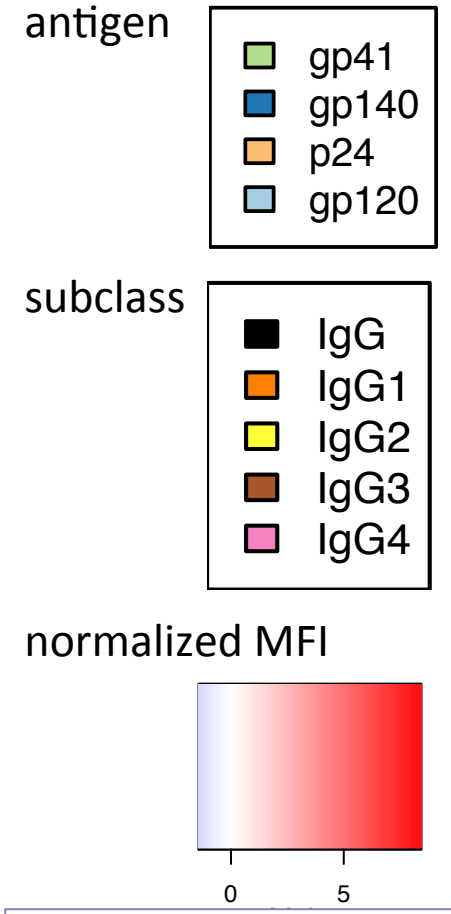






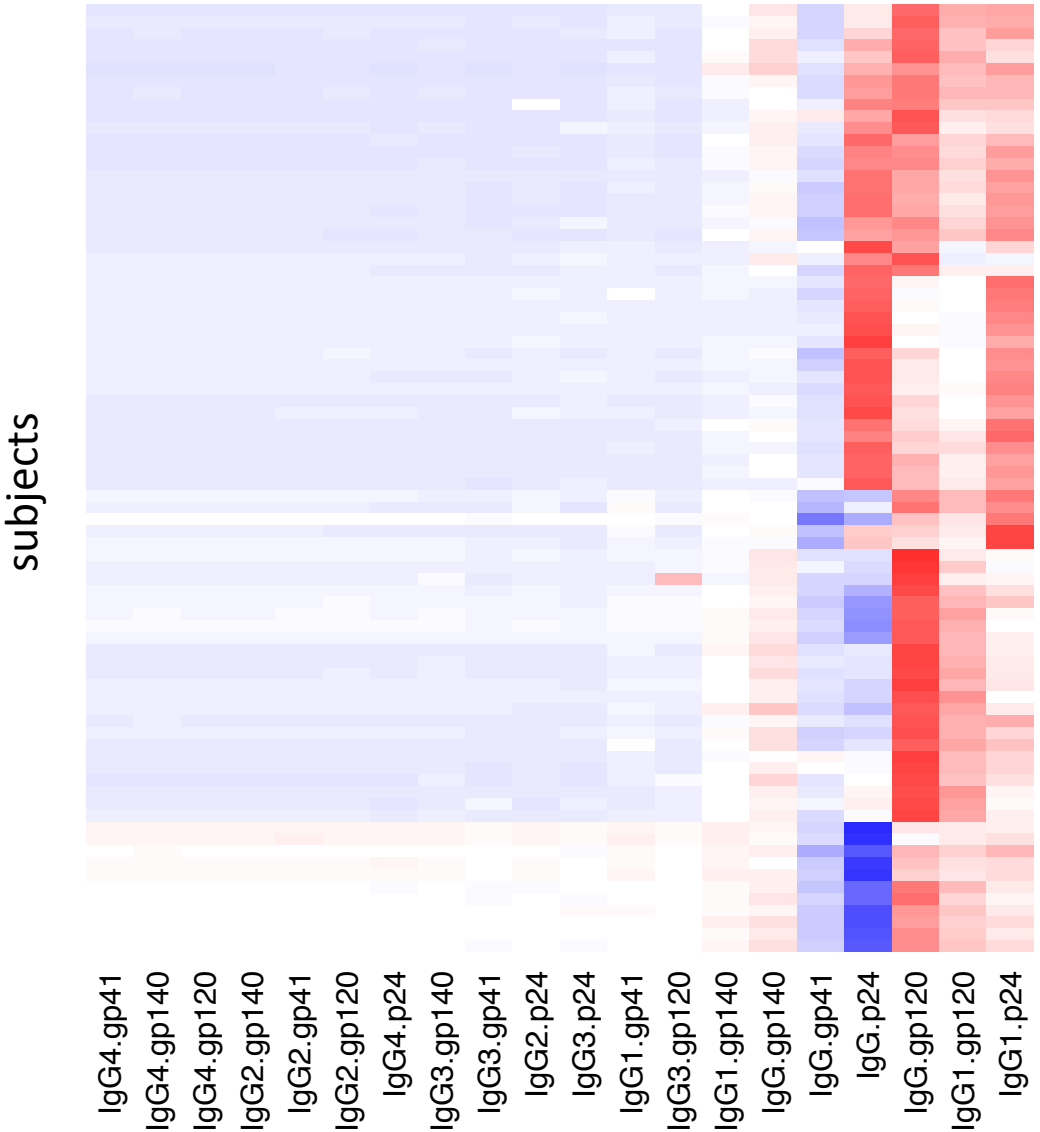
Similarities among features



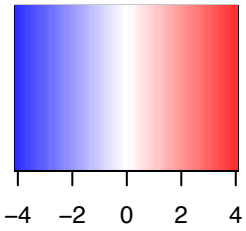


Clusters driven by combination of subclass and antigen

Similarities among subjects

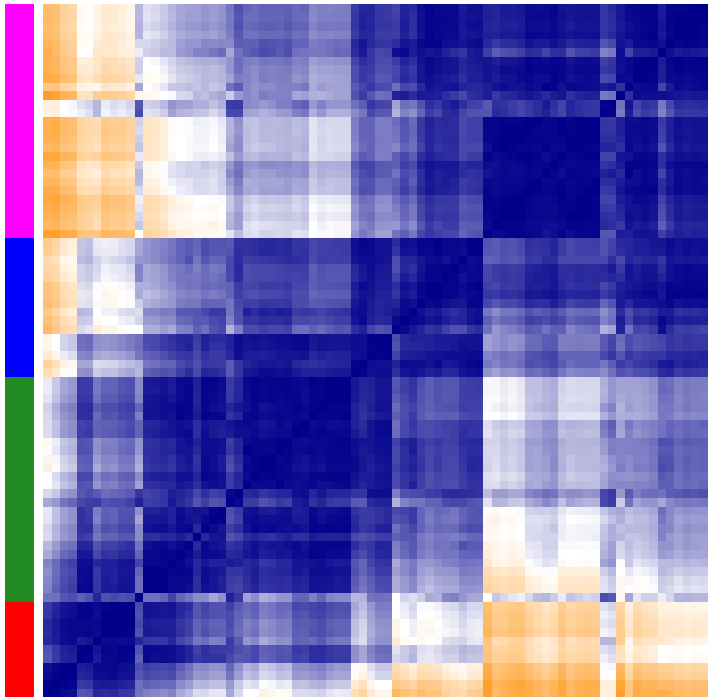


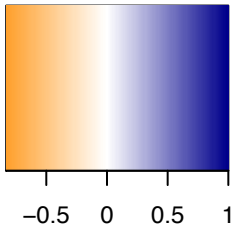
subject-normalized values
(slice of Ab pie per specificity)



Dominant p24 vs.
dominant gp120

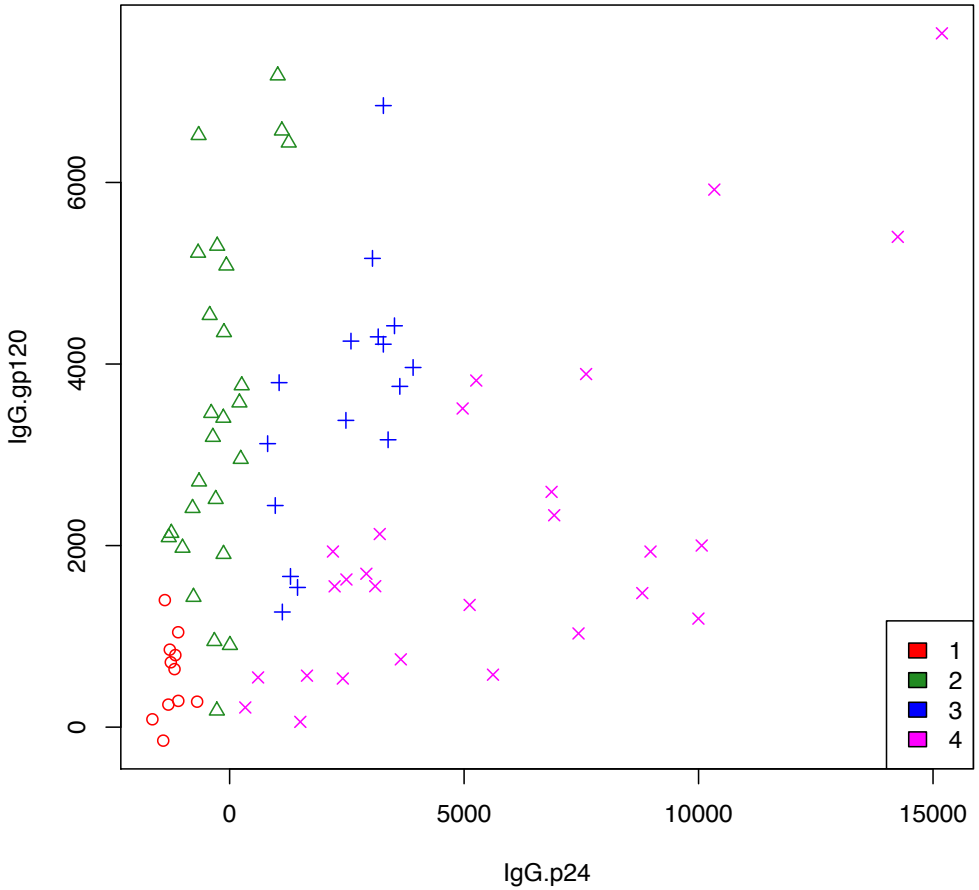
Subject-subject correlations, clustered



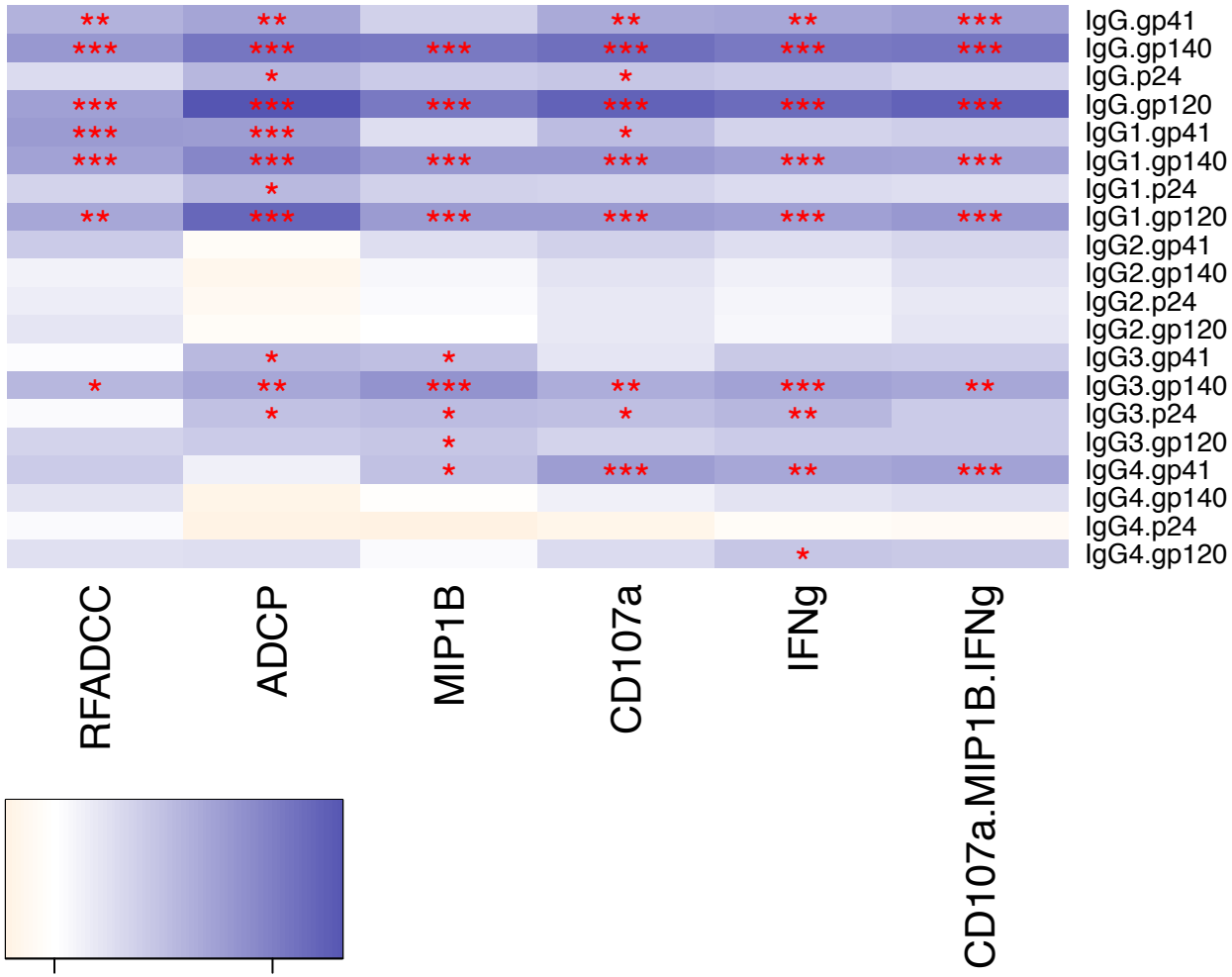


correlation coefficient

Their antigen-specific MFIs



Feature : function relationships

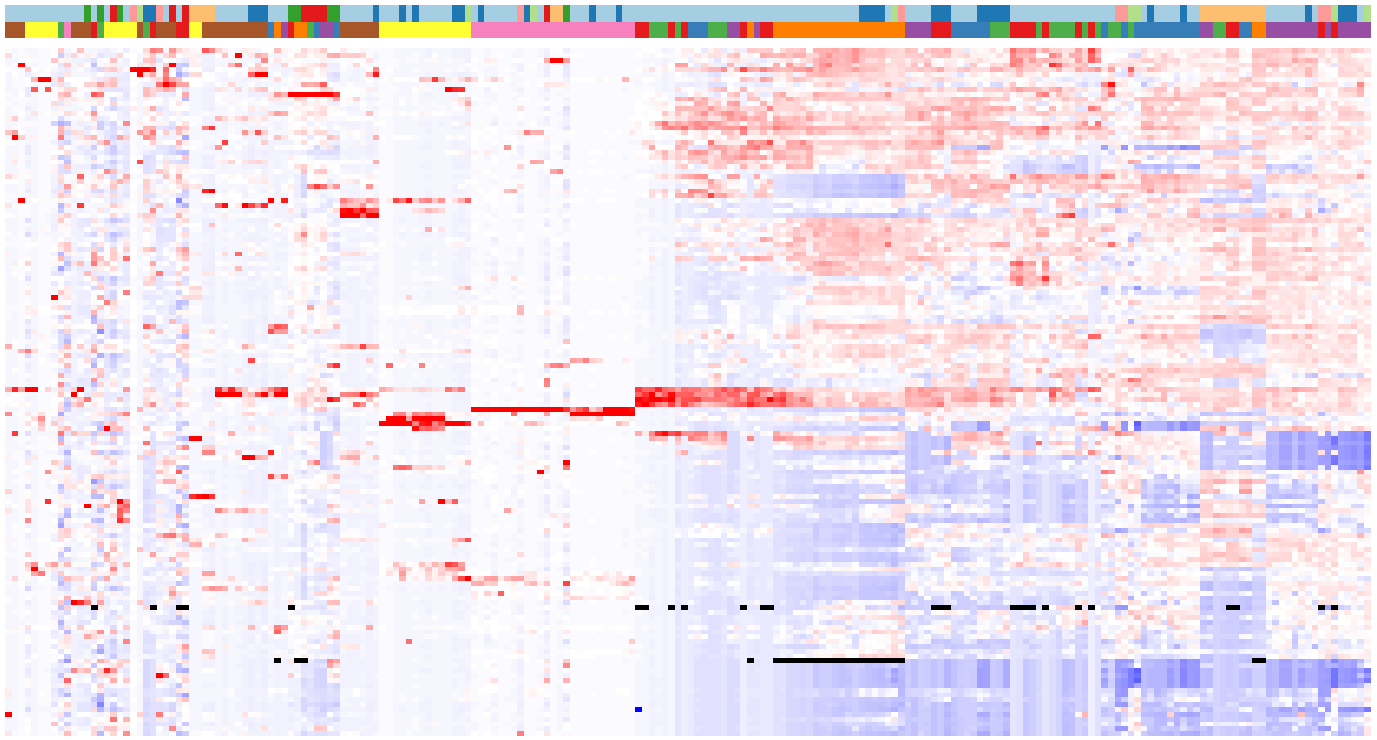


Features seem predictive

Antibody features

features

subjects



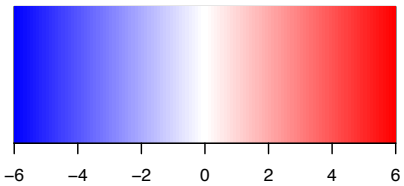
antigens

- gp120
- gp140
- gp41
- HIV1 IIIb Gag
- HIV1 Integrase
- HIV1 Vif (Ecoli)
- p24

readouts

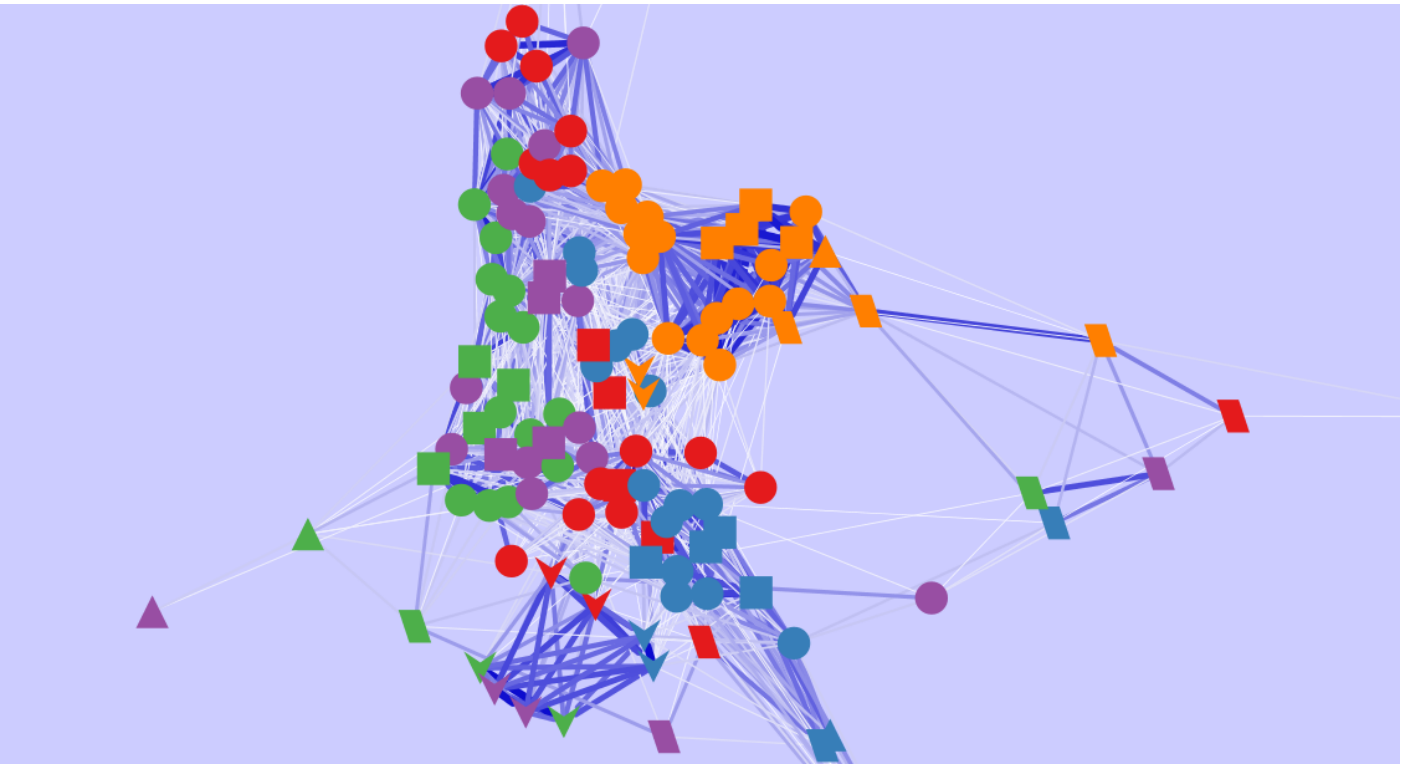
- C1q
- FcgRIIa
- FcgRIIb
- FcgRIIIa
- IgG1
- IgG2
- IgG3
- IgG4

normalized MFI



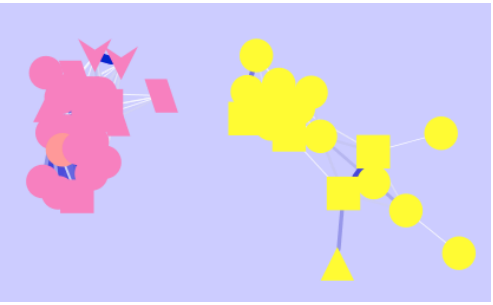
Clusters driven by combination of read-out and antigen

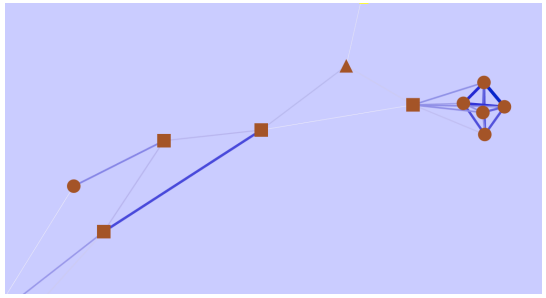
Another view



C1q
FcgRIIa
FcgRIIb
FcgRIIIa
IgG1
IgG2
IgG3
IgG4

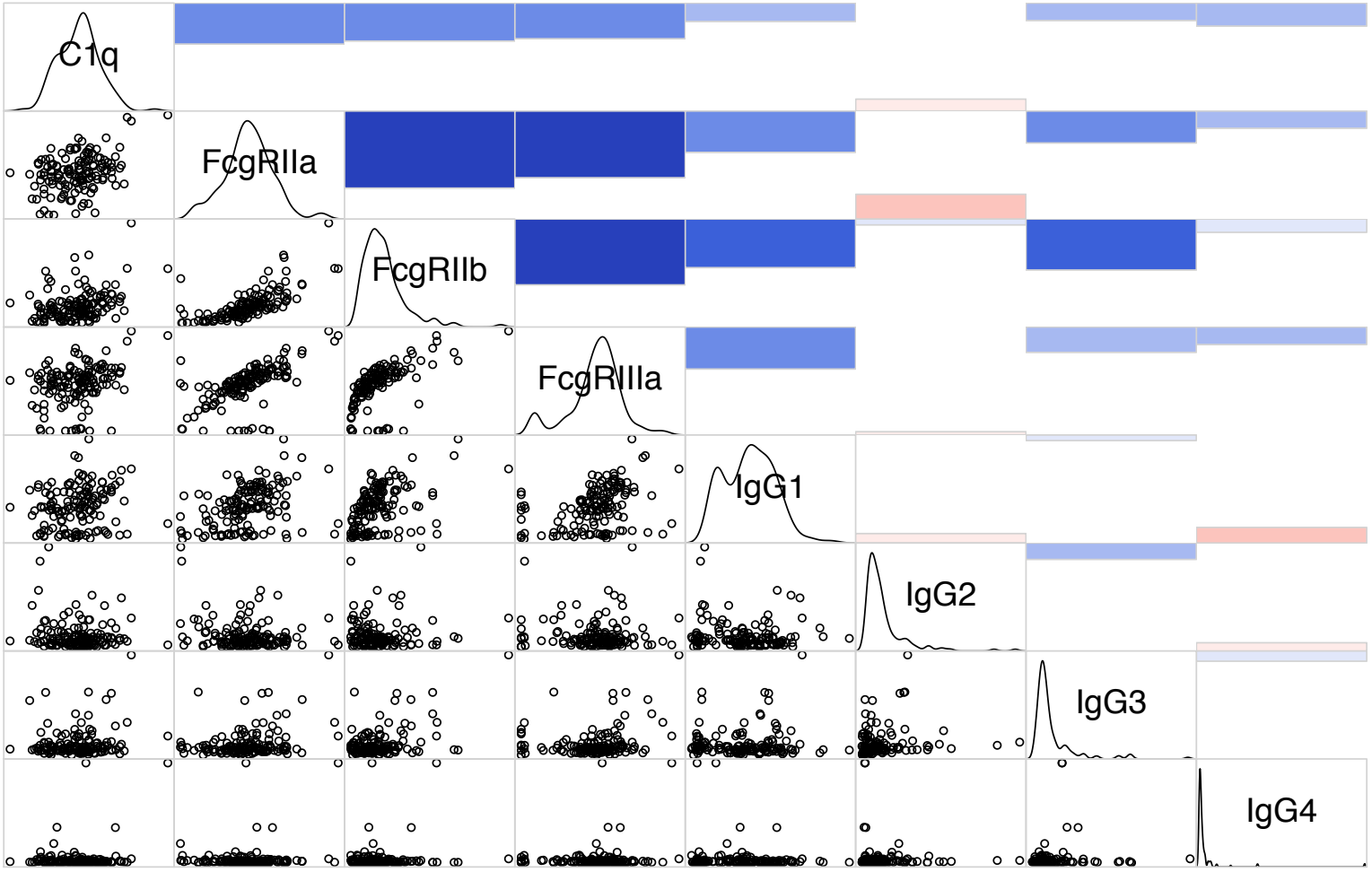
HIV1 IIIb Gag
HIV1 Integrase
HIV1 Vif (Ecoli)
gp120
gp140
gp41
p24





Clusters driven by combination of read-out and antigen

Particular feature relationships (for a particular gp120 antigen)

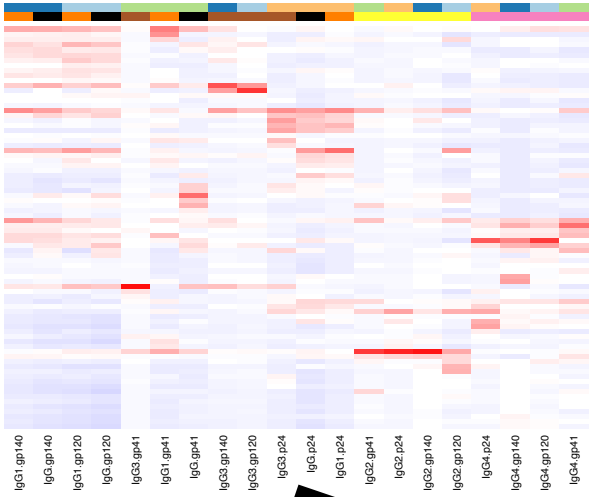


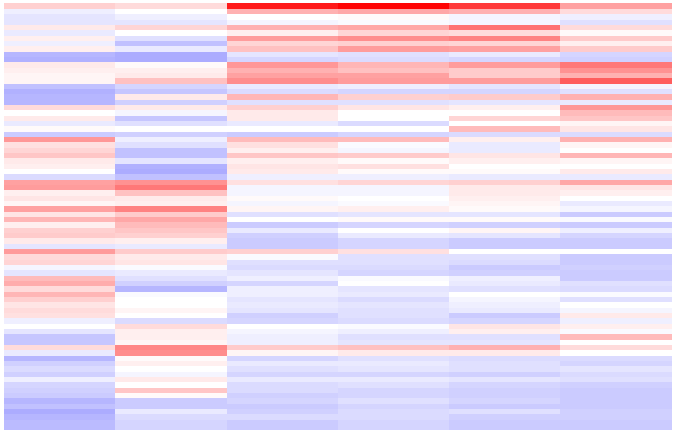
Correlations among FcgR, IgG1&3; bimodality

Supervised learning

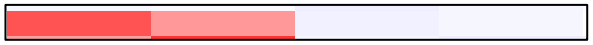
training: features

& corresponding functions





new subject

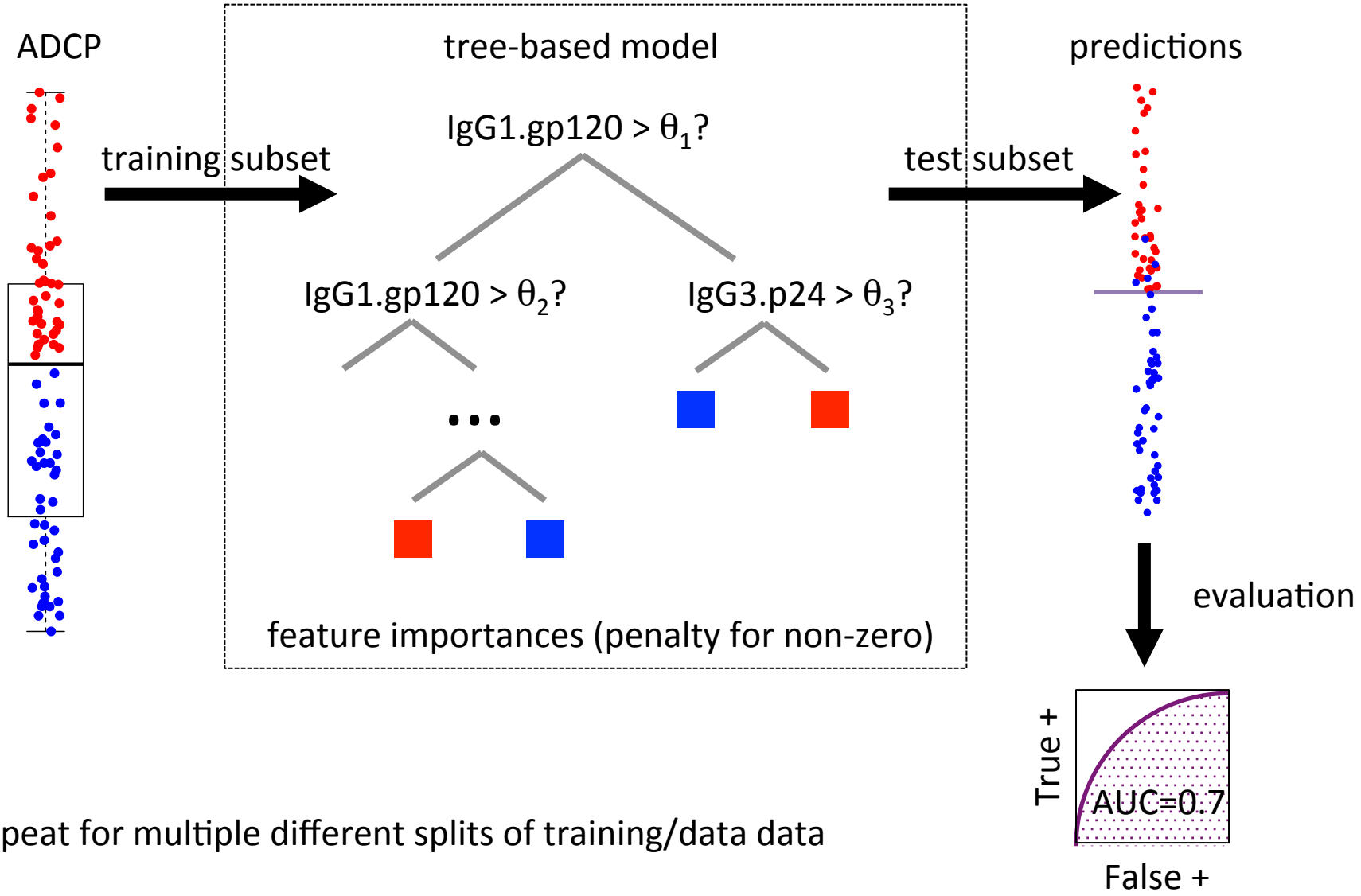


(subset of features)

predicted function

Lasso / logistic regression / random forest / support vector machine / Gaussian process

Classification: high vs. low function

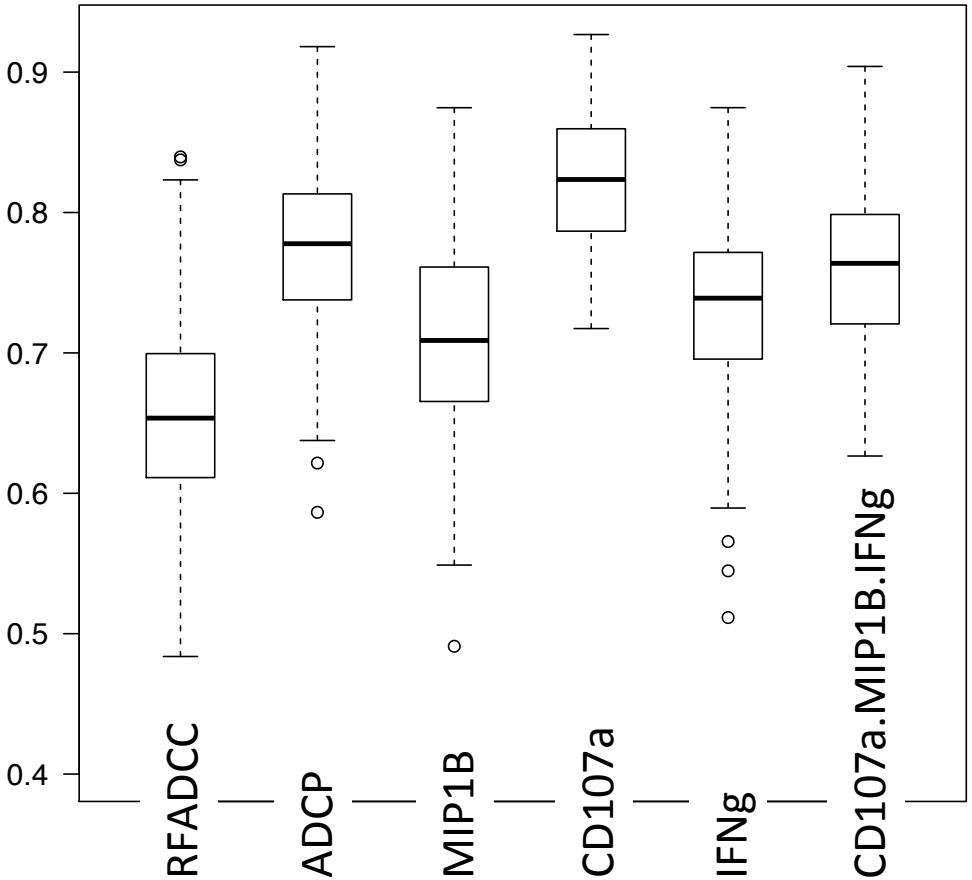


repeat for multiple different splits of training/data data

Classification example results

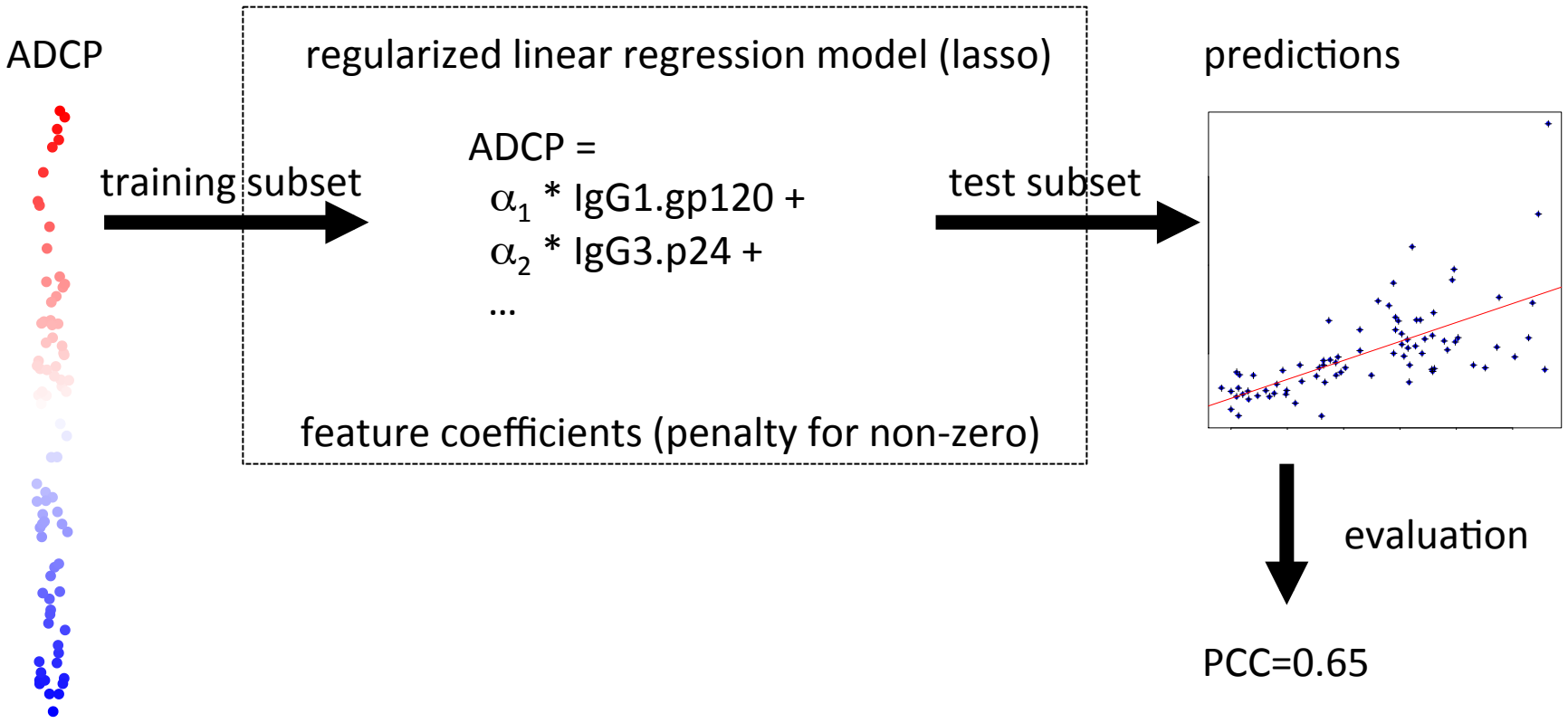
Regularized random forest; similar results with logistic- and kernel-based methods

AUC @
100-rep
2-fold CV



Models effectively use Ab features to qualitatively characterize functions

Regression: quantitative function

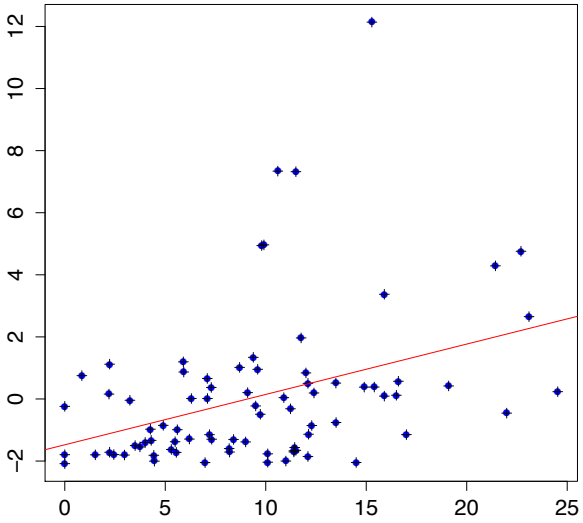


repeat for multiple different splits of training/data data

Regression example results

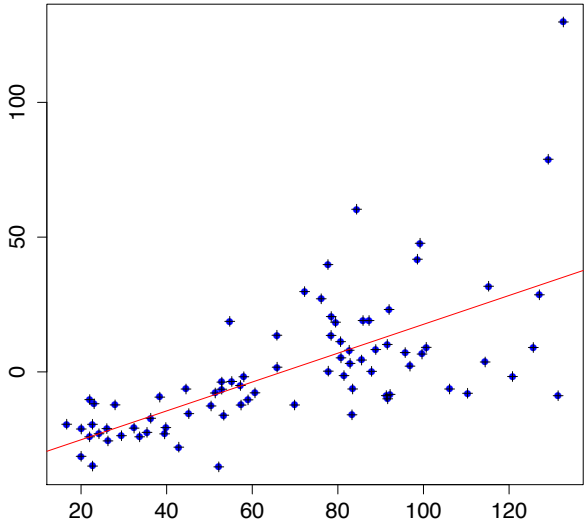
Lasso; similar results with tree- and kernel-based methods (LOOCV)

RFADCC



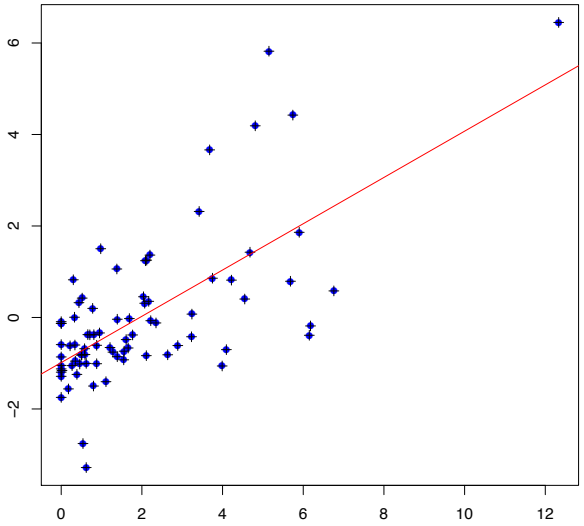
r = 0.37

ADCP



r = 0.65

CD107a.MIP1B.IFNg



r = 0.68

Models effectively use Ab features to quantitatively characterize functions