Can we use cellular markers to predict immunogenicity?

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WP2: Cellular characterization and mechanisms of the AD immune response

- WP2.1: To understand the cellular mechanisms causing AD responses
- WP2.2: To characterise ADA structurally and functionally
- WP2.3: To identify genetic markers predisposing to BP immunogenicity
- Patient cohorts:
  - **RA patients treated with TNF inhibitors (infliximab, adalimumab, etanercept), rituximab**
  - **IBD patients treated with TNF inhibitors**
  - **MS patients treated with IFNβ and/or natalizumab**
  - **HA patients treated with FVIII**
  - **SLE patients treated with rituximab**
- Considering including new BPs

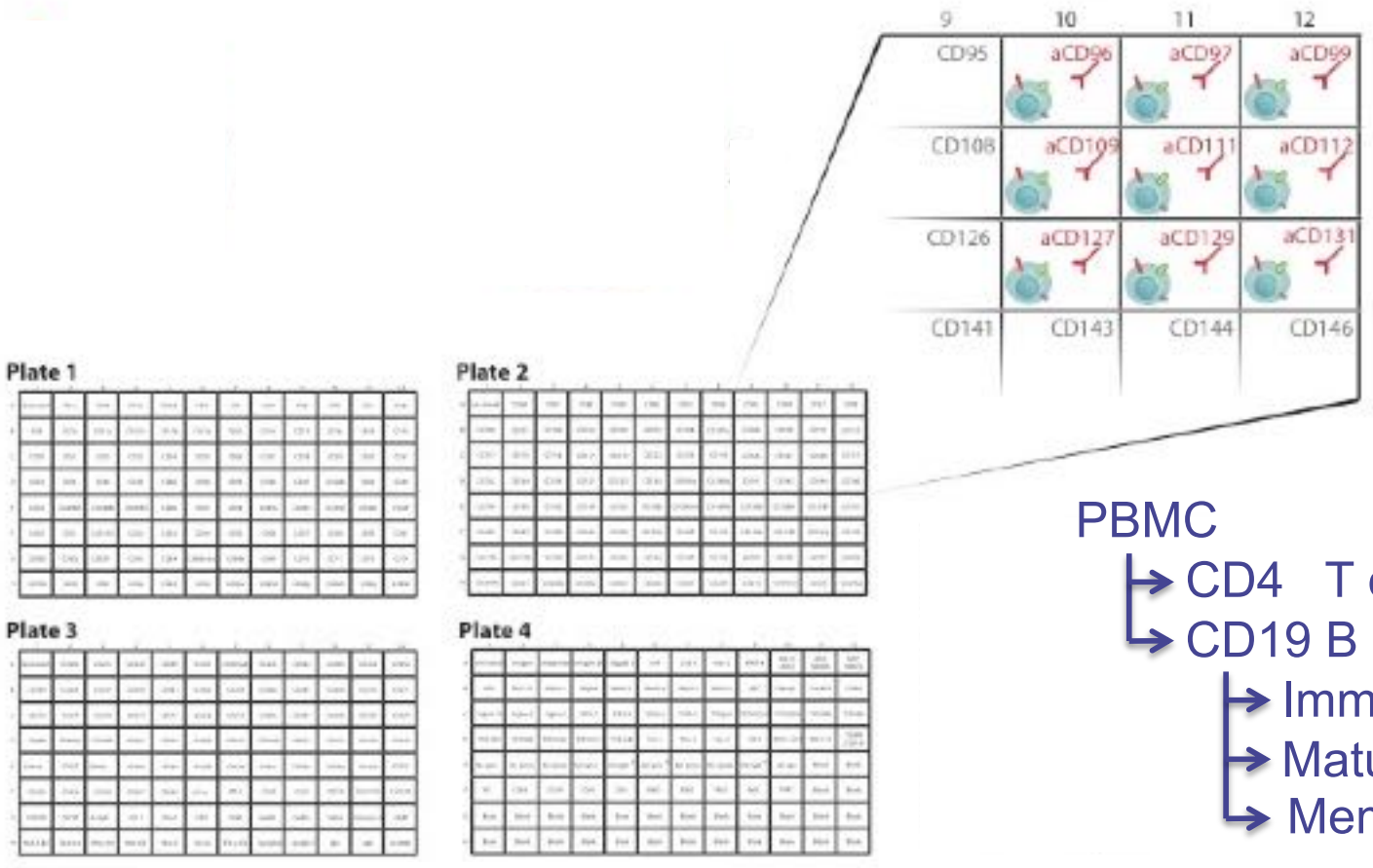
UCL WP2 objectives

- WP2.1.1: Evaluation of early activation biomarkers as potential predictors of immunogenicity
  - **Prospective: RA, MS, IBD**
  - **Cross sectional: RA, MS, SLE, IBD.**
- WP2.1.7 : Evaluation of B cell AD cellular response.
  - **Cross-sectional: RA, MS, IBD, HA**
- WP2.1.8 : Numerical and functional analysis of regulatory B cells in ADA+/ADA- patients
  - **Cross-sectional: pilot with RA, SLE then MS and HA**

Global immunophenotyping as a tool to investigate immunogenicity

- A new methodology
  - Validation with healthy donors
- Early Results
  - Studying B cell populations
  - Patients with MS
- Ongoing plans

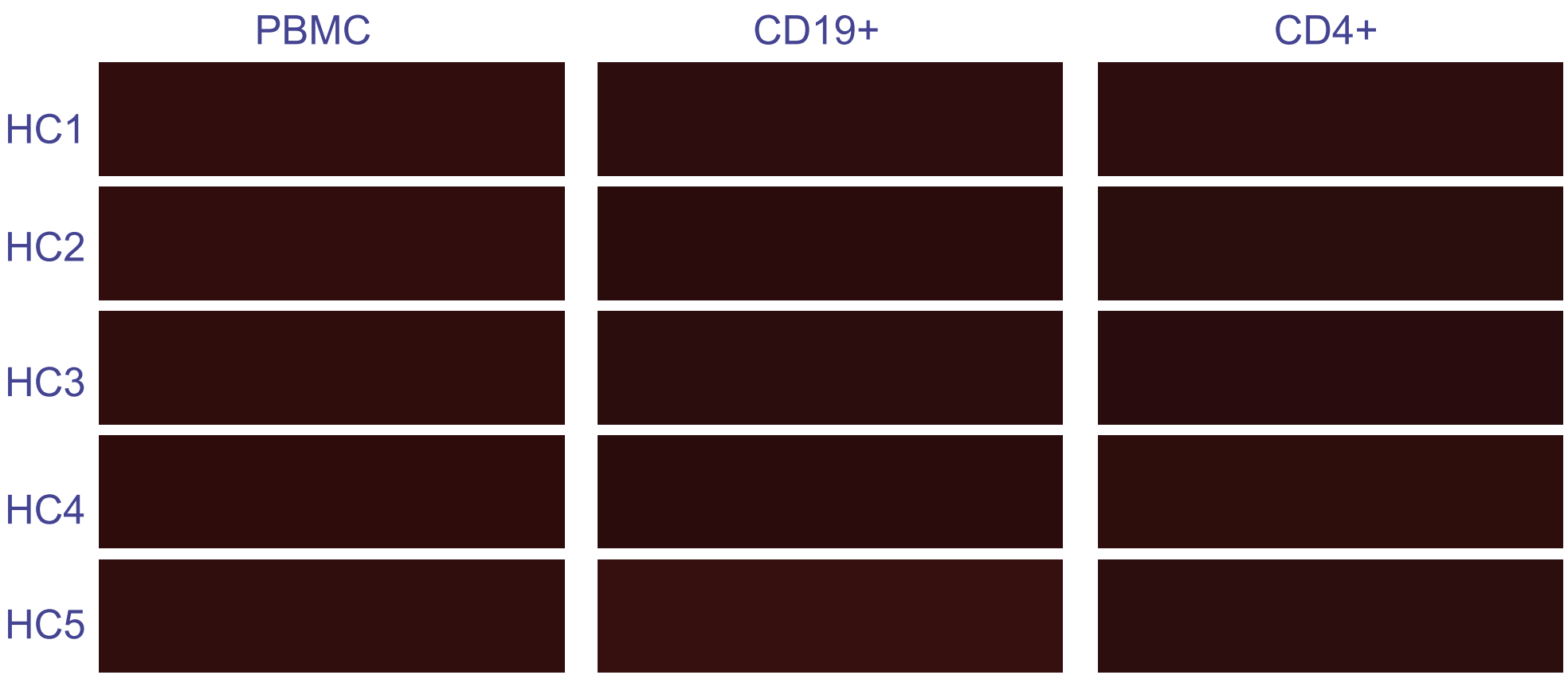
Novel flow cytometry platform: LEGENDScreen platform



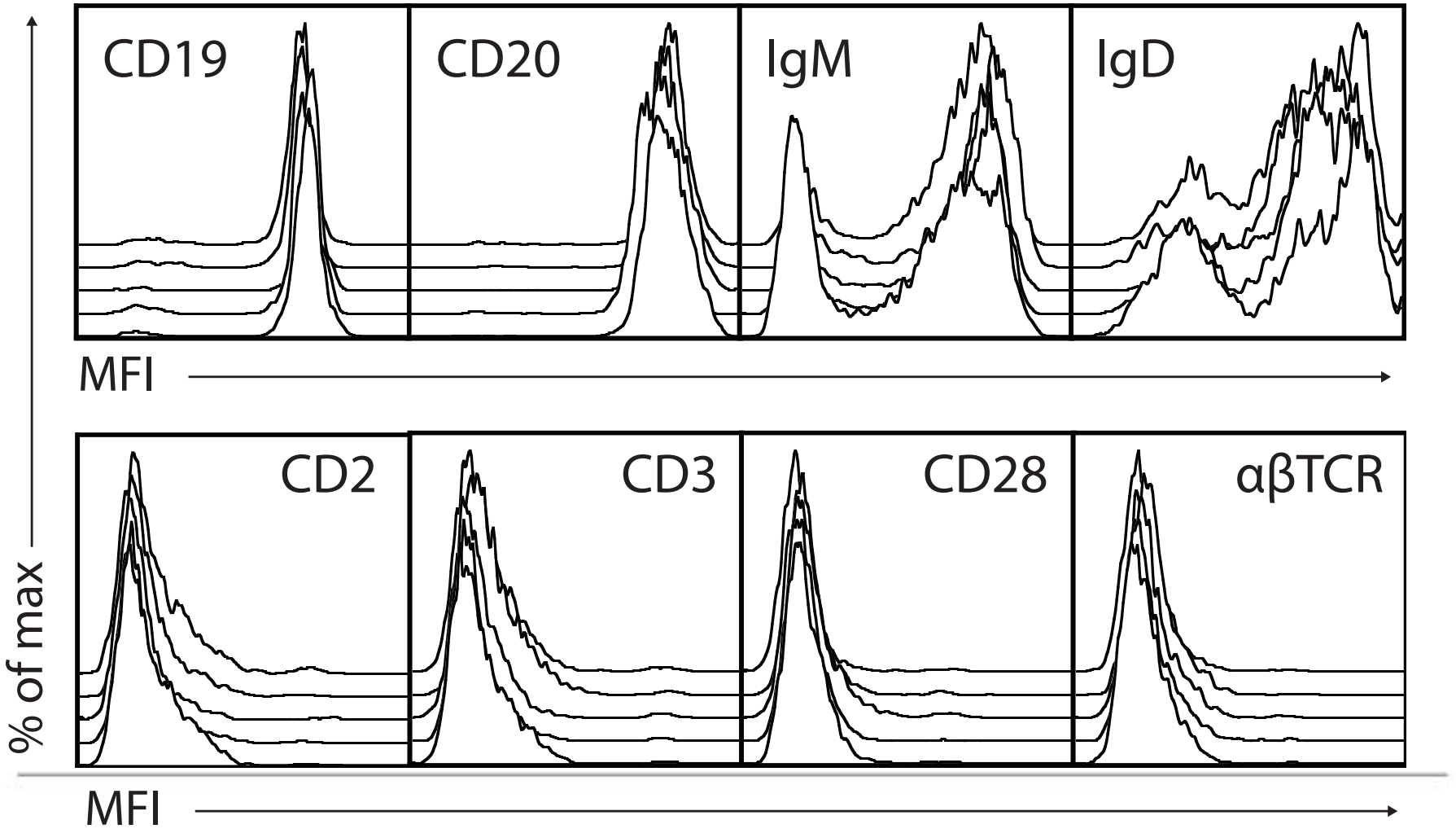
PBMC
- CD4 T cells
- CD19 B cells
  - Immature
  - Mature
  - Memory

332 CD antigens in parallel

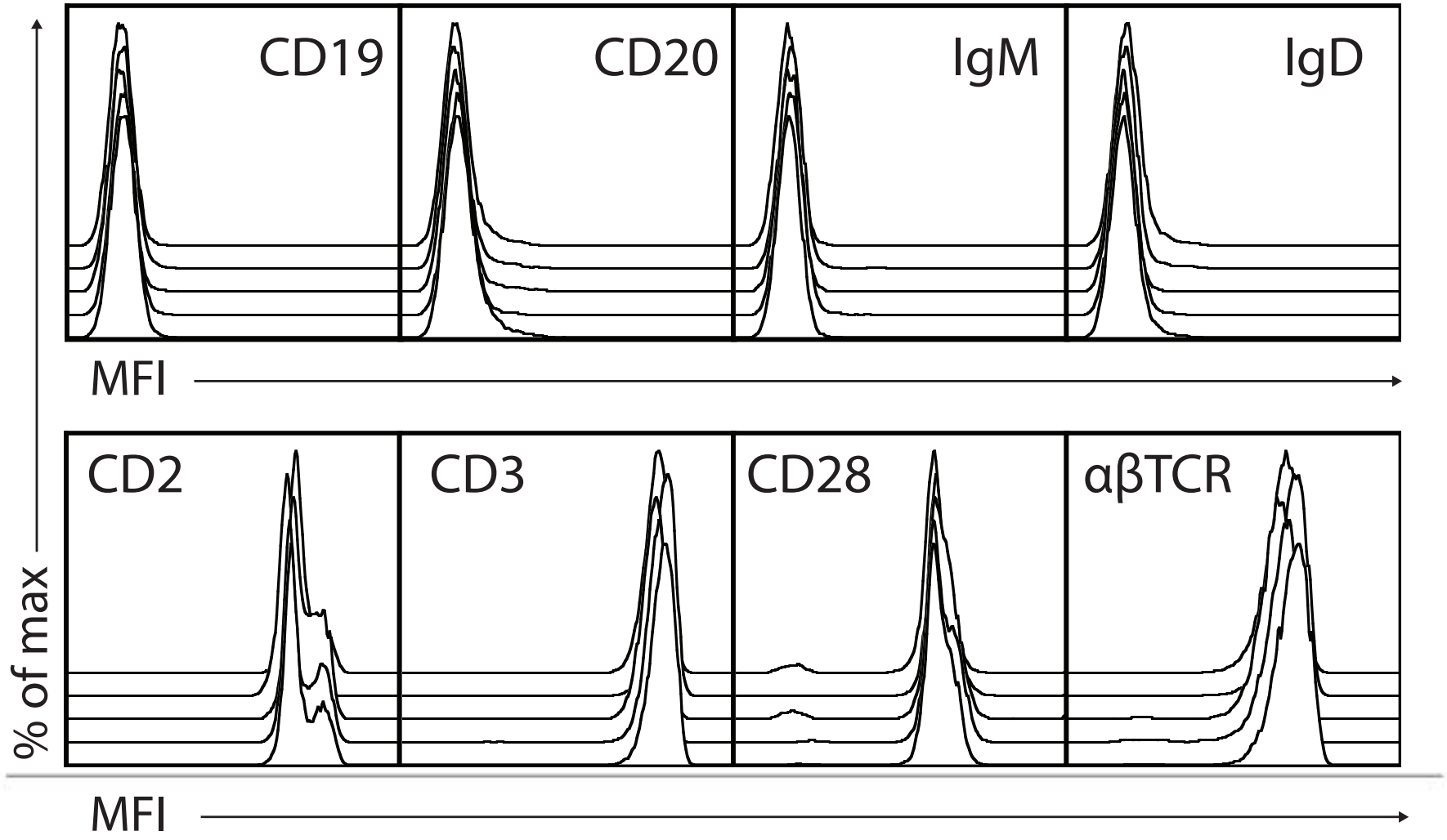
Validation: reproducibility



Validation: CD19+ gate



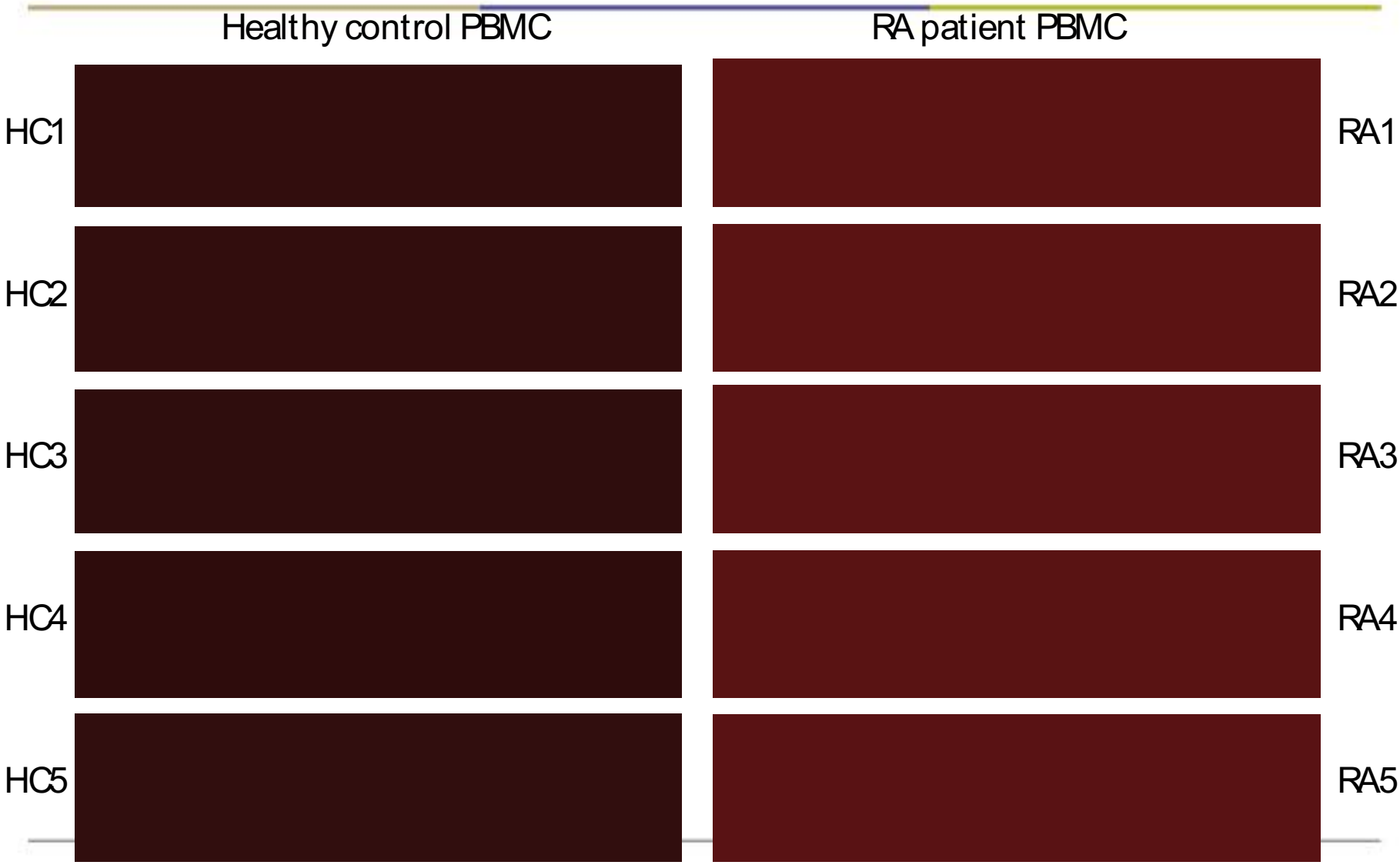
Validation: CD4+ gate



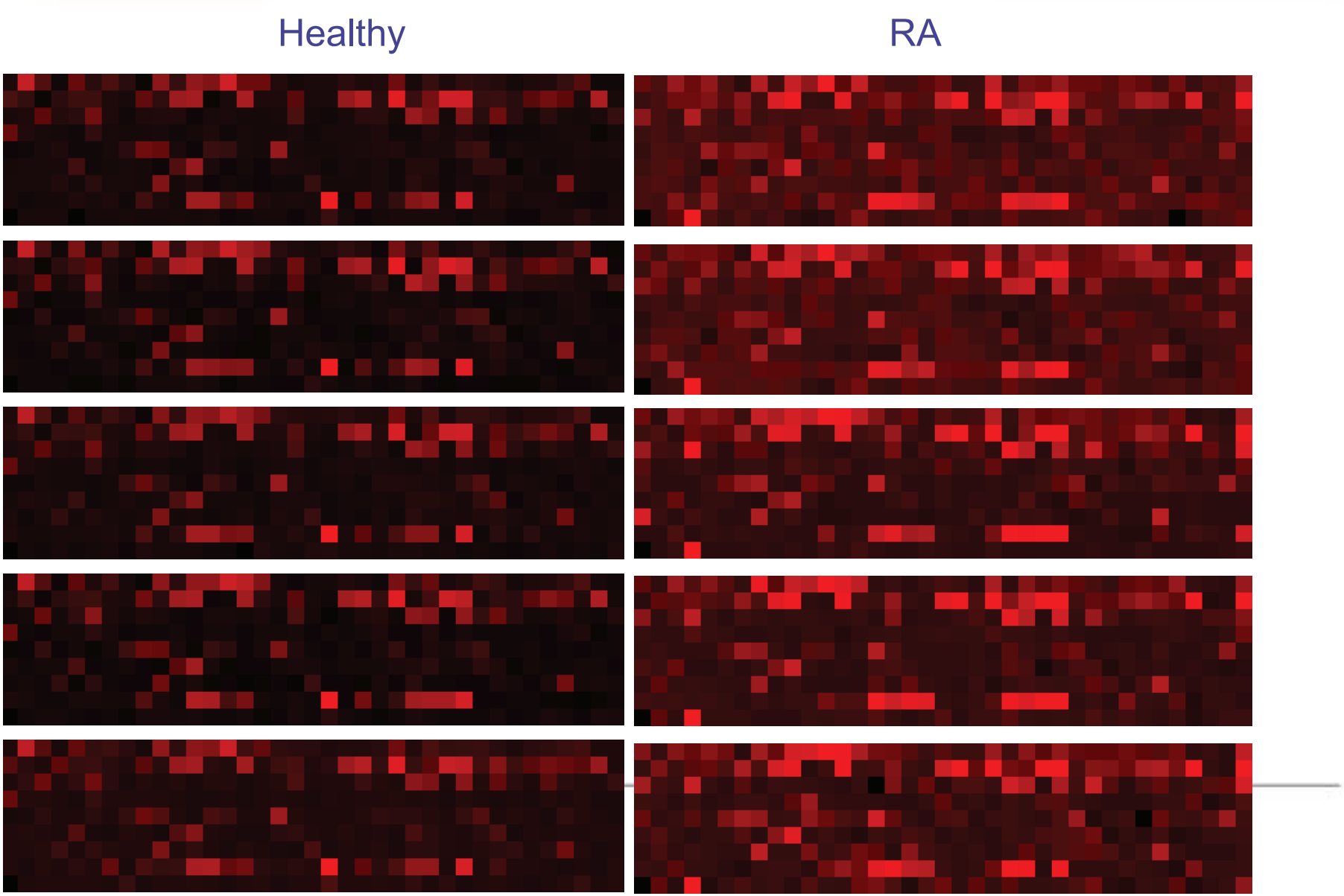
Healthy donors compared to RA patients

Age	Sex	Disease Activity Score	C-reactive Protein	Treatment
49	F	5.4	9.7	MTX HCQ
62	F	5.1	9.1	MTX SPZ
60	F	5.1	9.5	MTX SPZ
45	M	5.3	2.8	SPZ HCQ
61	F	5.6	10.7	SPZ

PBMC cell surface signature: Healthy vs RA



CD19^{+} profiling Healthy versus RA

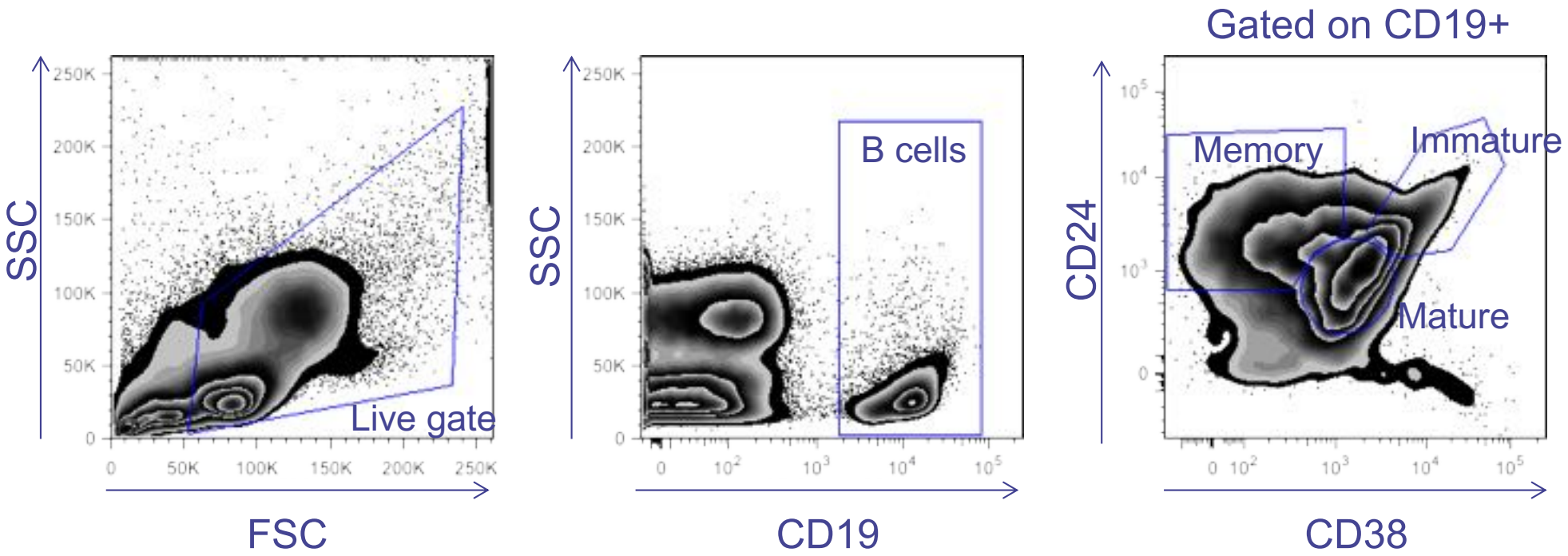


Global immunophenotyping as a tool to investigate immunogenicity

- A new methodology
  - Validation with healthy donors
- Early Results
  - **Studying B cell populations**
  - **Patients with RA, SLE and MS**
- Ongoing plans

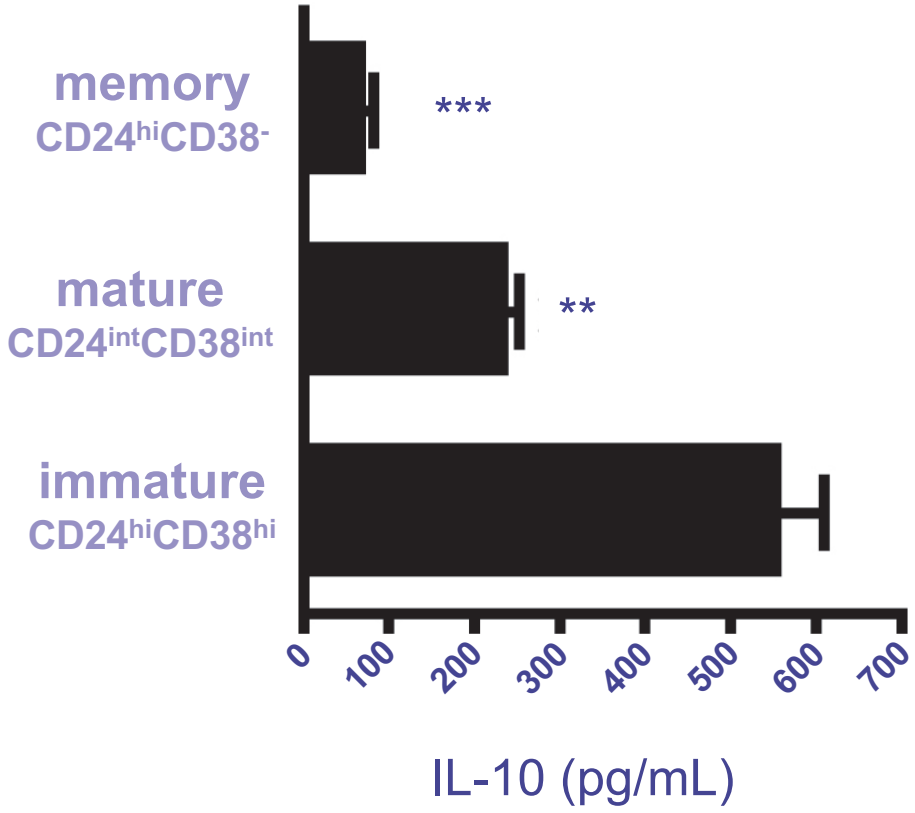
B cell subpopulations in PBMCs

Gating strategy



B cell subsets: Imm- Immature, Mat- Mature, Mem- Memory

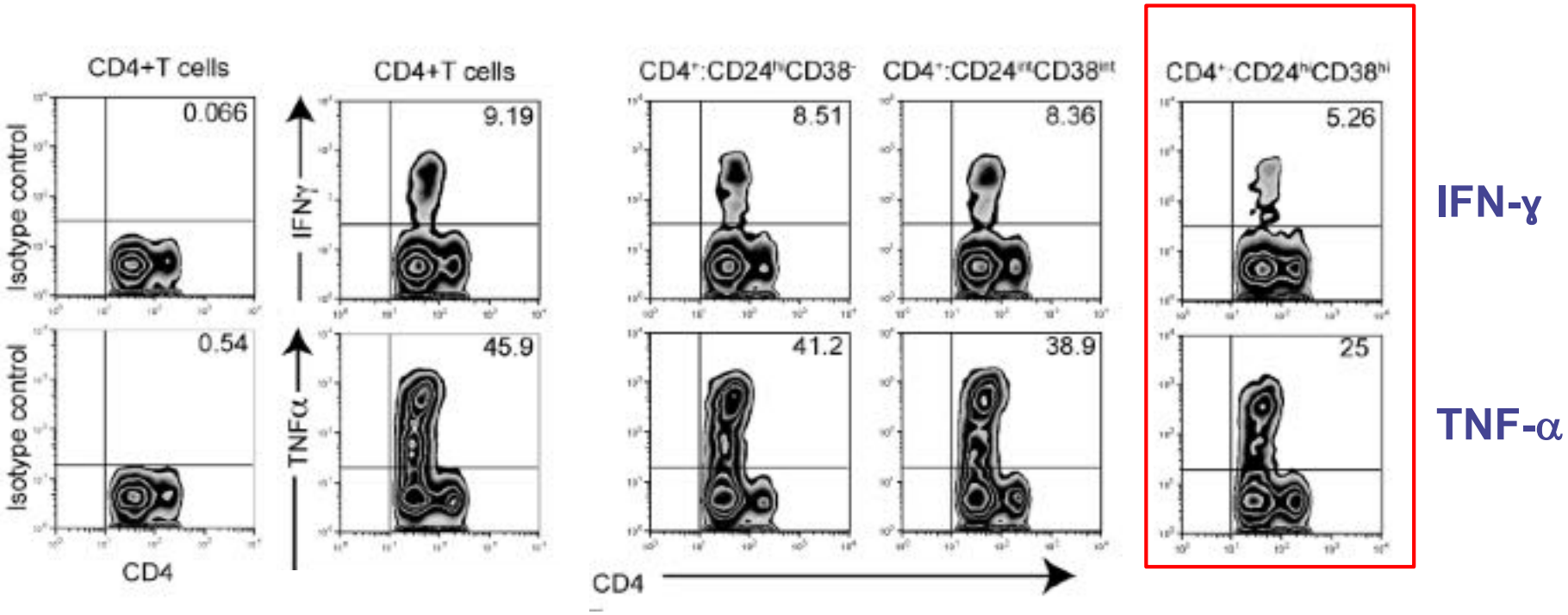
Immature B cells produce IL-10



IL-10 producing B cells are enriched in the $CD24^{hi}CD38^{hi}$ gate

Blair et al. *Immunity*; 2010

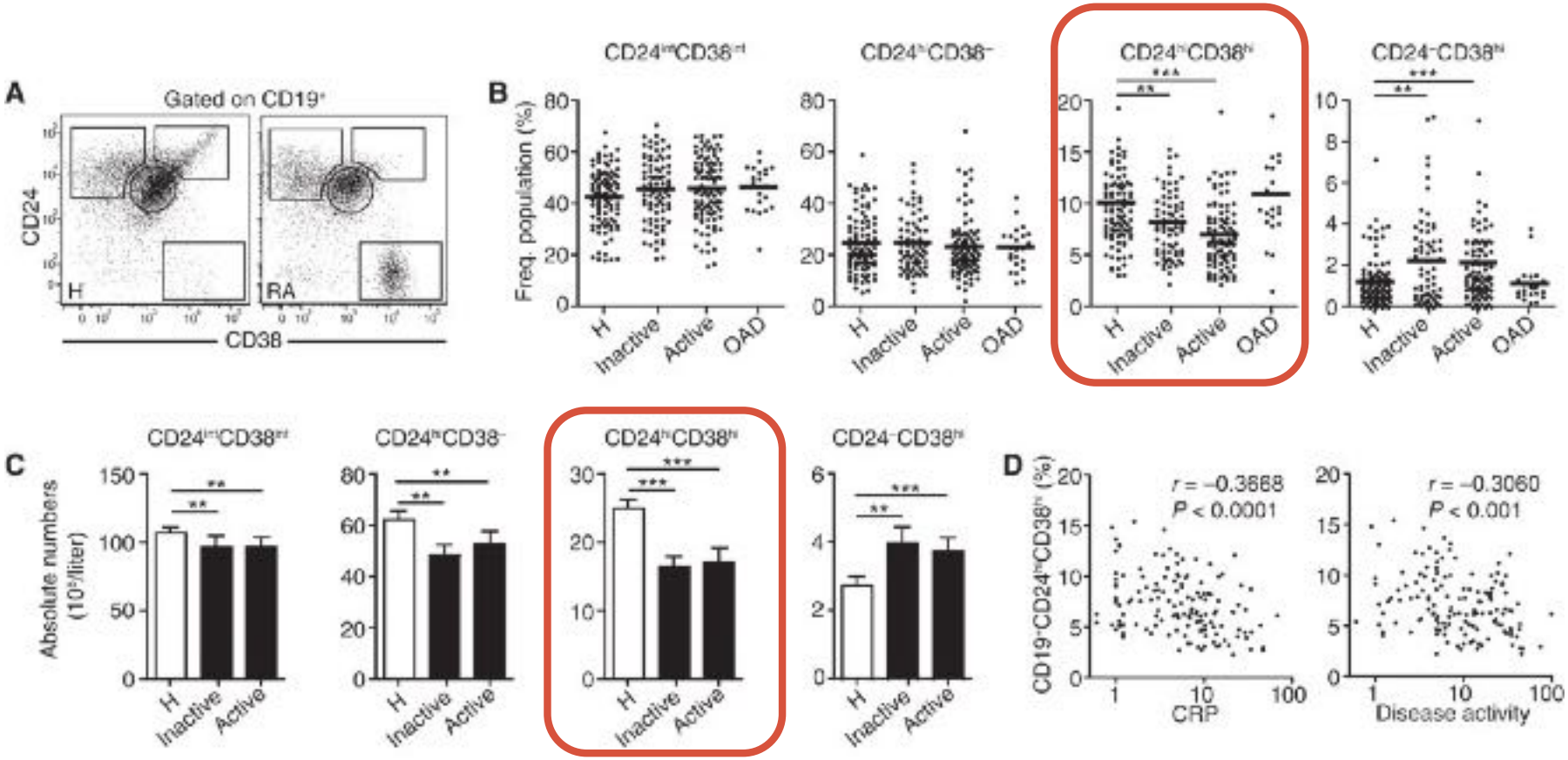
Immature B cells suppress T cells



$CD24^{hi}CD38^{hi}$ B cells suppress T helper cell differentiation

Blair et al. *Immunity*; 2010
Flores-Borja et al. *Science Trans Med*; 2013

B cell sub-populations
Relevance to Autoimmunity

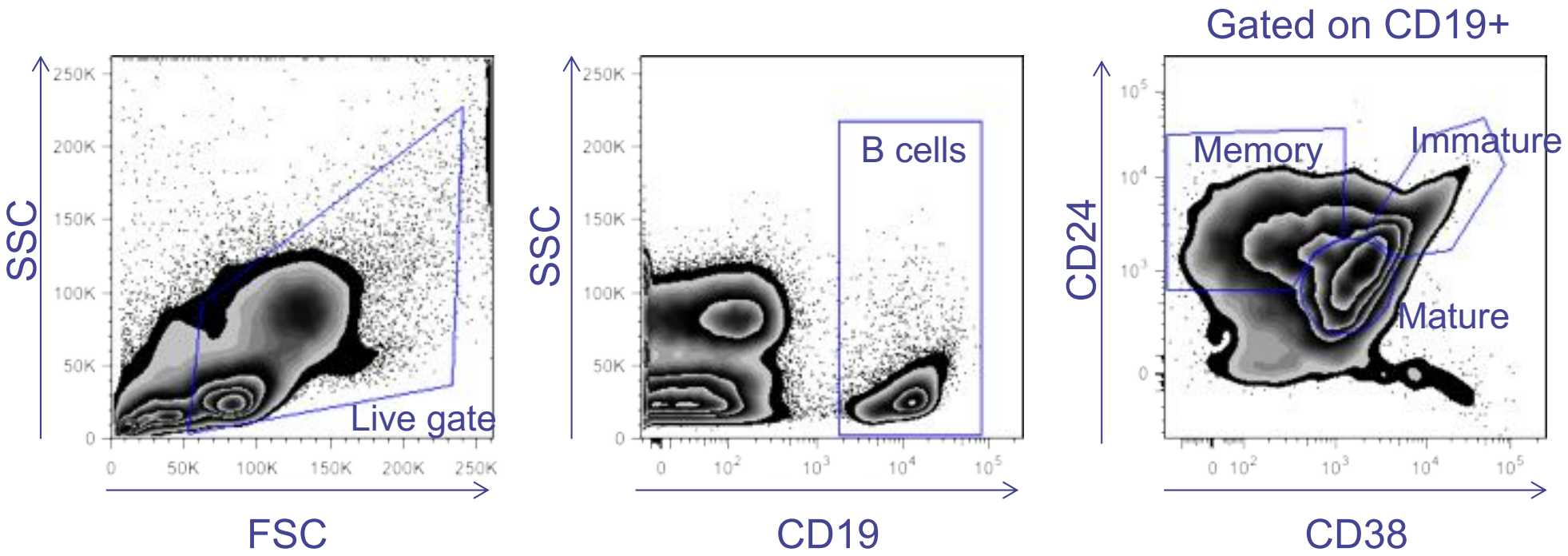


Number and frequency of $CD24^{hi}CD38^{hi}$ B cells are reduced in patients with active RA

Flores-Borja et al. *Science Trans Med*; 2013

LegendScreen gating strategy

Gating strategy

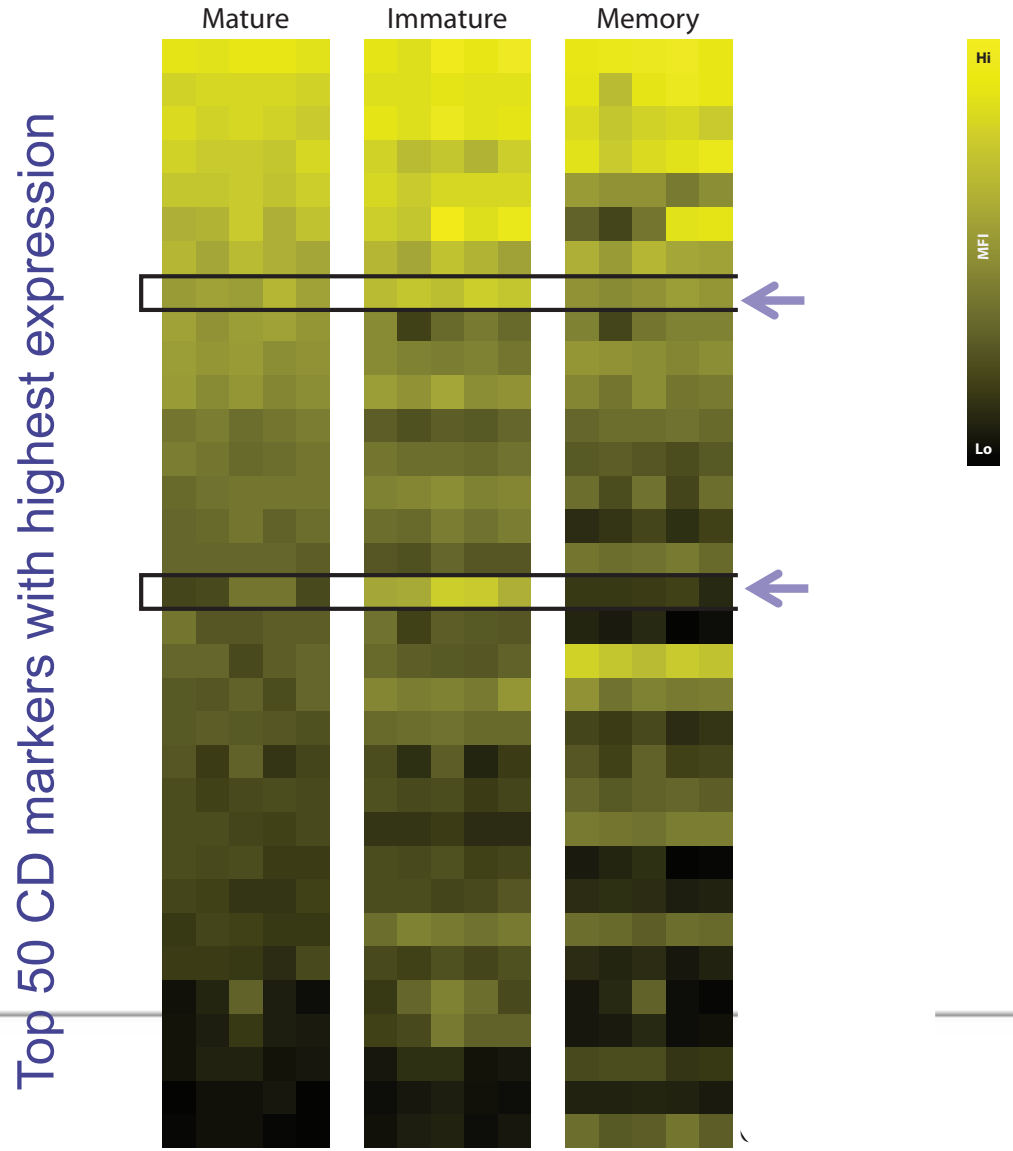


PLUS:
332 cell surface markers

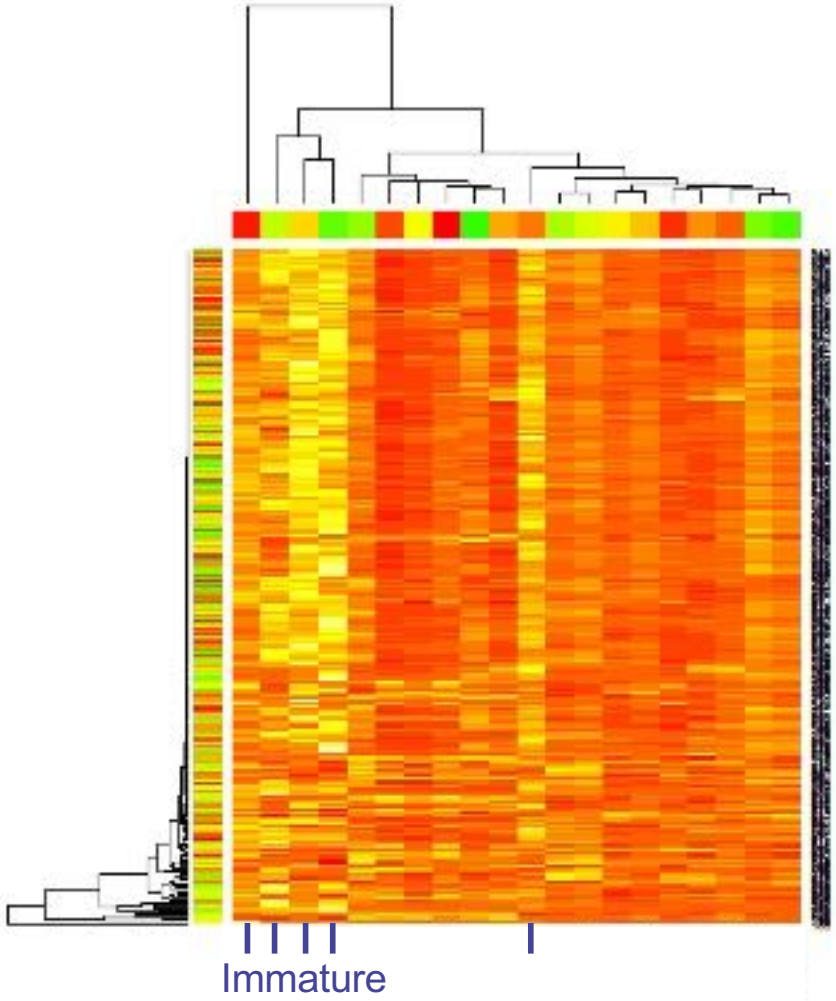
B cell subsets: Imm- Immature, Mat- Mature, Mem- Memory

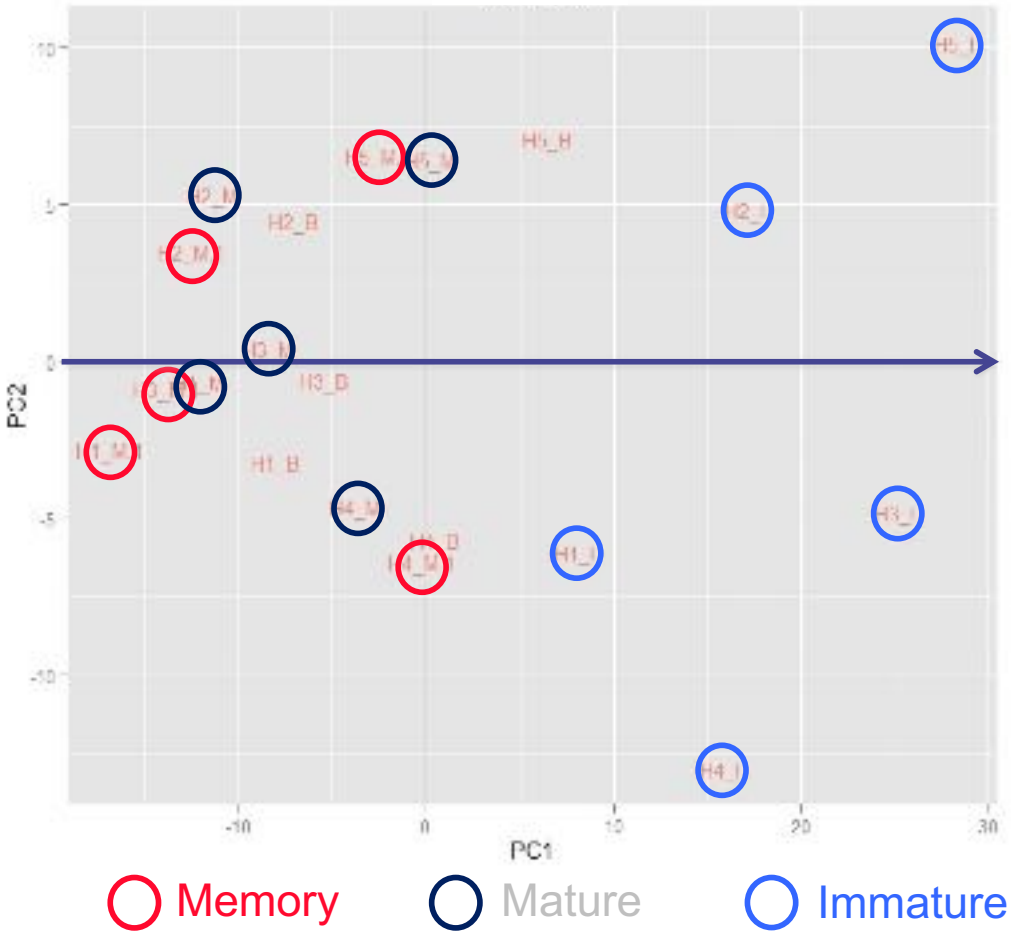
| 17

Results: B cell subsets have distinct expression profiles in healthy donors



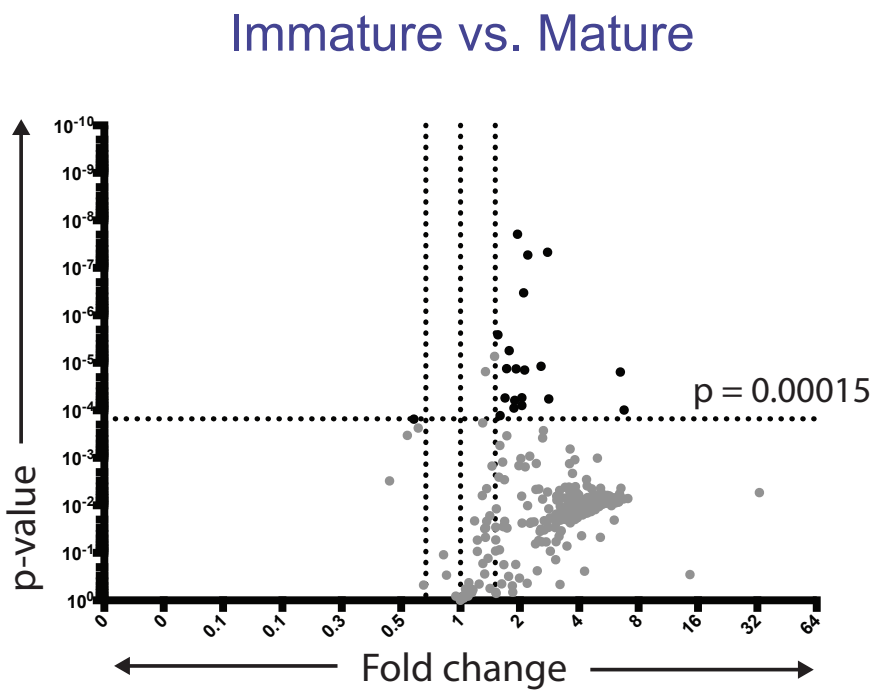
Comparing expression of 332 markers revealed that B cell subsets can be distinguished

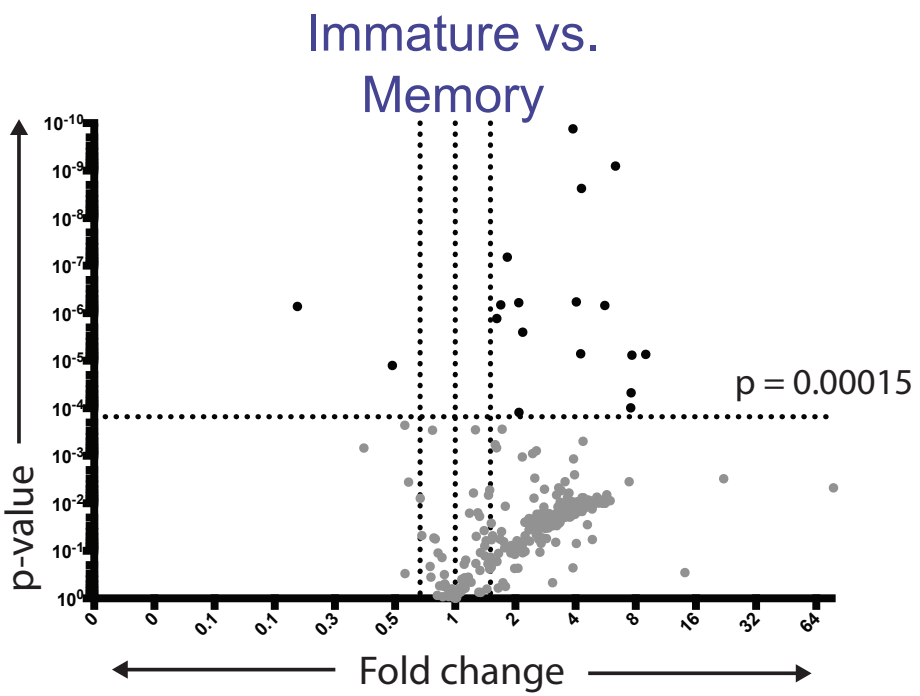




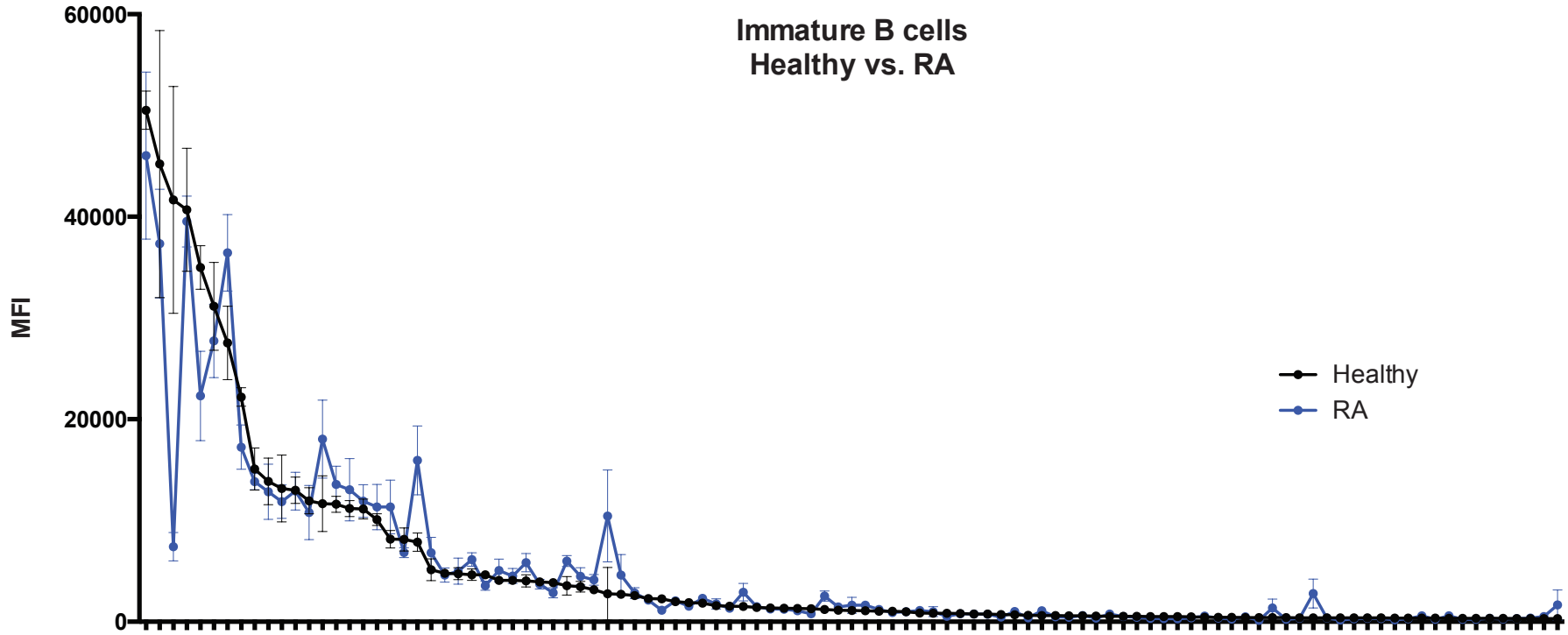
Pierre Dönnes: WP4

Specific markers show significantly altered expression in B cell subsets



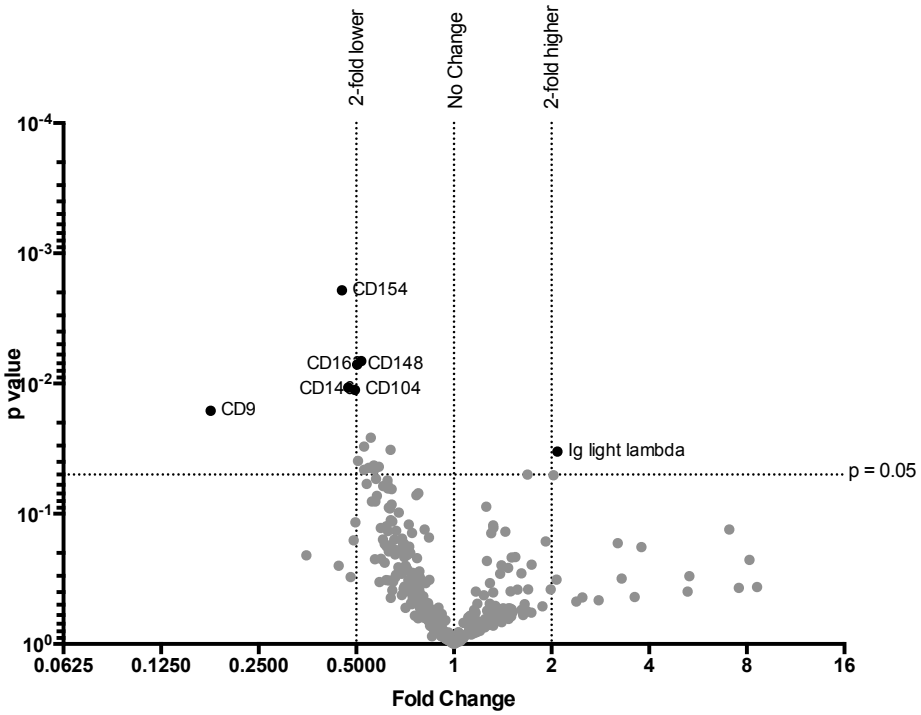


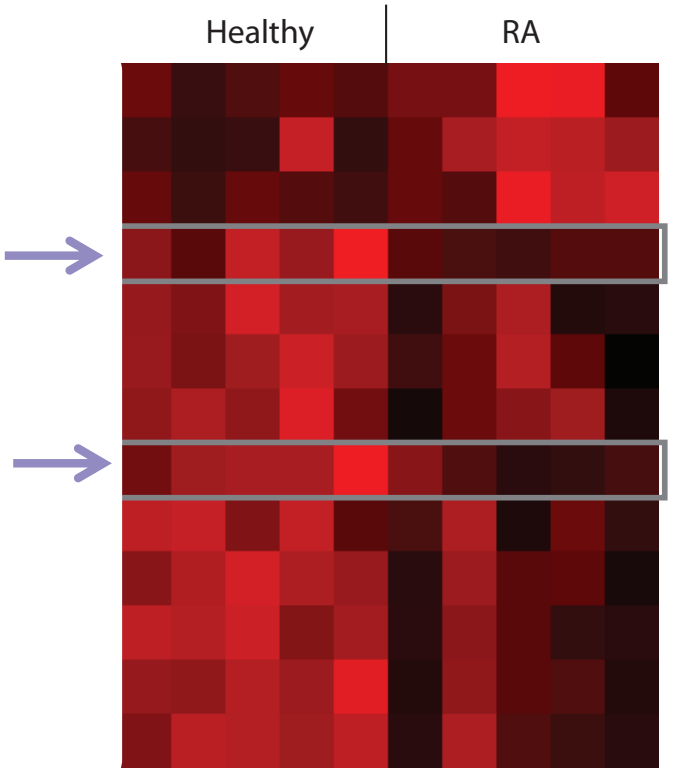
Comparison of Immature B cells from Healthy vs. RA patients revealed differential expression of some markers



Top 100 CD markers with highest expression

Several cell surface molecules have been identified as significantly different in RA compared to healthy immature B cells





Follow-up in ADA+ and ADA- patients

Summary 1

- Tool to define immature B cell phenotype
- Clinical tool to identify differences in B cell phenotype in healthy donors and RA patients
- Functional relevance of selected markers

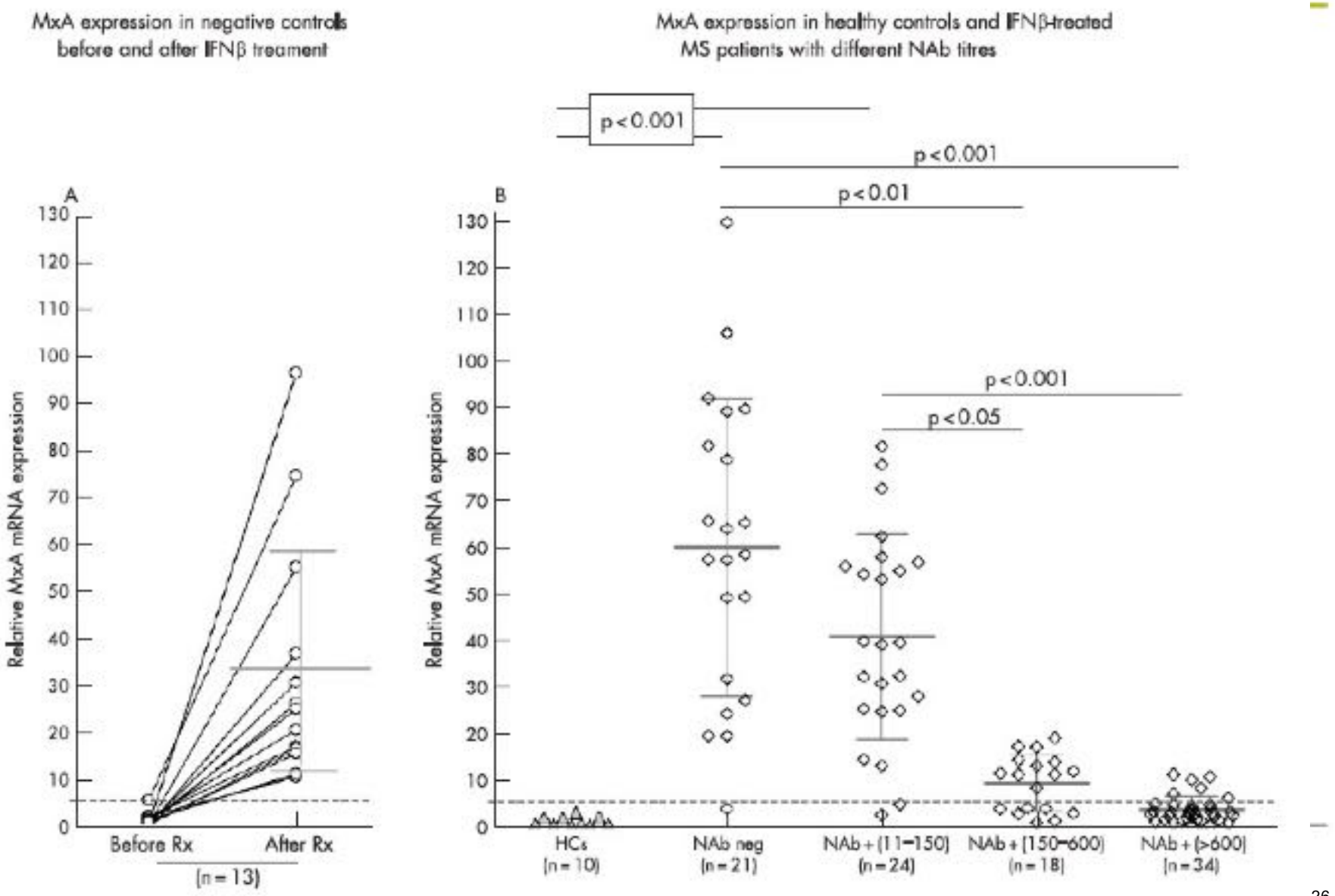
Global immunophenotyping as a tool to investigate immunogenicity

- A new methodology
  - Validation with healthy donors
- Early Results
  - Studying B cell populations
  - **Patients with MS (ADA+ vs ADA-)**
- Ongoing plans

Differential Immunophenotype of ADA+ and ADA- MS patients

- 32 MS patient's (14 Male & 18 Female)
  - **10 CIS (clinically isolated syndrome)**
  - **22 RRMS (remitting relapsing)patients**
- EDSS (expanded disability status) between 1 and 4 (mean 2.09)
- Average age: 38.5 ± 9.5 years
- 22 treated with IFN-β 1a (11 Avonex™;11 Rebif™)
- 10 treated with IFN-β 1b (6 Extavia™; 4 Betaferon™)
- Blood sampled 10-14h after last IFN-β injection
- 18 Assayed for MxA expression (13 high, 2 middle, 3 low)
- Neutralizing Abs determination: 11 positive / 25 assayed (44% of tested)
- Neutralizing Abs titre: unknown

Rational for sample selection



Sominanda et al 2008 JNNP

ABIRISK Number	Gender	Birth Date	Time between last injection and blood sampling (hours)	MS Course	Date of onset	EDSS	Medication	MxA	NABs
Abirisk 008	M	23/12/1960	14	RR MS	15/03/2011	2.5	Avonex	High MxA	ND
Abirisk 009	M	09/12/1969	12	CIS	15/10/2006	4	Extavia	Low MxA	ND
Abirisk 013	F	09/02/1977	12	RR MS	15/06/1998	1.5	Betaferon	Low MxA	ND
Abirisk 023	M	18/11/1967	11	RR MS	15/04/2011	2	Avonex	High MxA	NABs Neg

Cell Populations of Interest

- Peripheral blood Tfh cells
  - Phenotype: CD4+CXCR5+:
  - IgM, IgG and IgA secretion (IL-21 & ICOS dependent);
  - B cell proliferation (IL-21 & ICOS dependent);
  - B cell differentiation in CD38+CD19lo plasmablasts;

- Peripheral blood CD19+ B cells
  - Subpopulations phenotype:
  - CD19+CD24hiCD38hi (Transitional B cells- Bregs)
  - CD19+CD24intCD38int (Mature B cells)
  - CD19+CD24hiCD38- (Memory B cells)

- Myeloid Derived Suppressor Cells (MDSCs)
  - Phenotype: CD14+DR-/lo
  - Reported to both suppress or enhance the autoimmune response in EAE

LegendScreen™

ABIRISK

Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	CD1a	CD1b	CD1c	CD1d	CD2	CD3	CD4	CD5	CD6	CD7	CD8a
B	CD9	CD10	CD11a	CD11b	CD11b (activated)	CD11c	CD13	CD14	CD15	CD16	CD18	CD19
C	CD20	CD21	CD22	CD23	CD24	CD25	CD26	CD27	CD28	CD29	CD30	CD31
D	CD32	CD33	CD34	CD35	CD36	CD38	CD39	CD40	CD41	CD42b	CD43	CD44
E	CD45	CD45RA	CD45RB	CD45RO	CD46	CD47	CD48	CD49a	CD49c	CD49d	CD49e	CD49f
F	CD50 (ICAM-3)	CD51	CD51/61	CD52	CD53	CD54	CD55	CD56 (NCAM)	CD57	CD58	CD59	CD61
G	CD62E	CD62L	CD62P (P-Selectin)	CD63	CD64	CD66a/c/e	CD66b	CD69	CD70	CD71	CD72	CD73
H	CD79b	CD80	CD81	CD82	CD83	CD84	CD85a (ILT5)	CD85d (ILT4)	CD85g (ILT7)	CD85h (ILT1)	CD85j (ILT2)	CD85k (ILT3)

Plate 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	CD220	CD221 (IGF-1R)	CD226 (DNAM-1)	CD229 (Ly-9)	CD231 (TALLA)	CD235ab	CD243	CD244 (2B4)	CD245 (p220/240)	CD252 (OX40L)	CD253 (Trail)
B	CD254	CD255 (TWEAK)	CD257 (BAFF)	CD258 (LIGHT)	CD261 (DR4)	CD262 (DR5)	CD263 (DcR1)	CD266 (Fn14)	CD267 (TACI)	CD268 (BAFF-R)	CD270 (HVEM)	CD271
C	CD273 (B7-DC)	CD274 (B7-H1)	CD275 (B7-H2)	CD276	CD277	CD278 (ICOS)	CD279 (PD-1)	CD282 (TLR2)	CD284 (TLR4)	CD286 (TLR6)	CD292	CD294
D	CD298	CD300e (IREM-2)	CD300F	CD301	CD303	CD304	CD305	CD307d (FcRL4)	CD314 (NKG2D)	CD317	CD318 (CDCP1)	CD319 (CRACC)
E	CD324 (E-Cadherin)	CD325	CD326 (Ep-CAM)	CD328 (Siglec-7)	CD334 (FGFR4)	CD335 (NKp46)	CD336 (NKp44)	CD337 (NKp30)	CD338 (ABCG2)	CD340 (erbB2)	CD344 (Frizzled-4)	CD351
F	CD352 (NTB-A)	CD354 (TREM-1)	CD355 (CRTAM)	CD357 (GITR)	CD360 (IL-21R)	β2-micro-globulin	BTLA	C5aR	C5L2	CCR10	CLEC12A	CLEC9A
G	CX3CR1	CXCR7	δ-Opioid Receptor	DLL1	DLL4	DR3 (TRAMP)	EGFR	erbB3/HER-3	FcεRIα	FcRL6	Galectin-9	GARP (LRRC32)
H	HLA-A,B,C	HLA-A2	HLA-DQ	HLA-DR	HLA-E	HLA-G	IFN-γ R β chain	Ig light chain κ	Ig light chain λ	IgD	IgM	IL-28RA

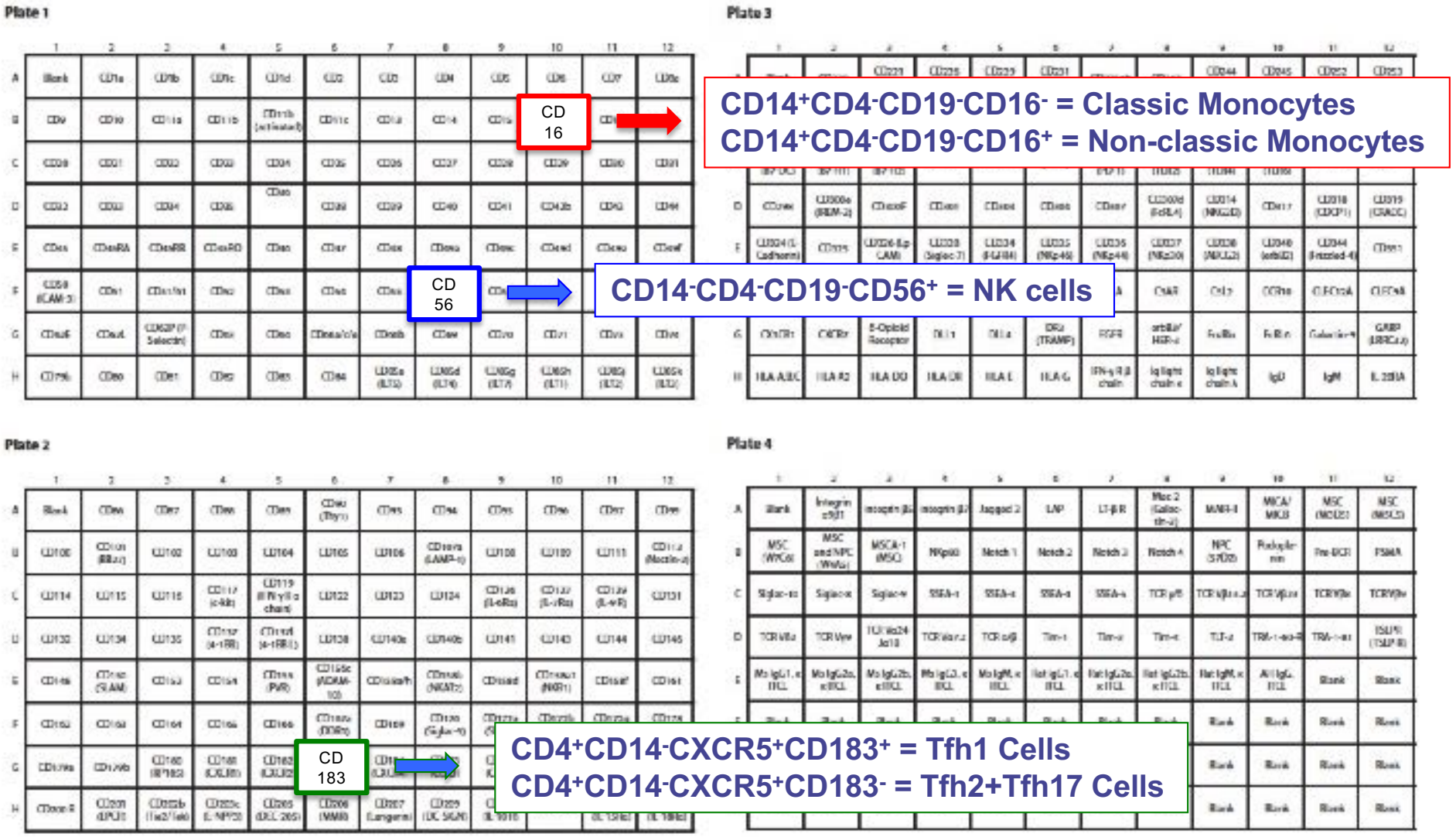
Plate 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	CD86	CD87	CD88	CD89	CD90 (Thy1)	CD93	CD94	CD95	CD96	CD97	CD98
B	CD100	CD101 (BB27)	CD102	CD103	CD104	CD105	CD106	CD107a (LAMP-1)	CD108	CD109	CD111	CD112 (Nectin-2)
C	CD114	CD115	CD116	CD117 (c-kit)	CD119 (IFN-γ R α chain)	CD122	CD123	CD124	CD126 (IL-6Rα)	CD127 (IL-7Rα)	CD129 (IL-9R)	CD131
D	CD132	CD134	CD135	CD137 (4-1BB)	CD137L (4-1BB L)	CD138	CD140a	CD140b	CD141	CD143	CD144	CD146
E	CD148	CD150 (SLAM)	CD152	CD154	CD155 (PVR)	CD156c (ADAM-10)	CD158a/h	CD158b (NKAT2)	CD158d	CD158e1 (NKB1)	CD158f	CD161
F	CD162	CD163	CD164	CD165	CD166	CD167a (DDR1)	CD169	CD170 (Siglec-5)	CD172a (SIRPα)	CD172b (SIRPβ)	CD172g (SIRPγ)	CD178 (Fas-L)
G	CD179a	CD179b	CD180 (RP105)	CD181 (CXCR1)	CD182 (CXCR2)	CD183	CD184 (CXCR4)	CD193 (CCR3)	CD195 (CCR5)	CD196	CD197 (CCR7)	CD200 (OX2)
H	CD200 R	CD201 (EPCR)	CD202b (Tie2/Tek)	CD203c (E-NPP3)	CD205 (DEC-205)	CD206 (MMR)	CD207 (Langerin)	CD209 (DC-SIGN)	CD210 (IL-10R)	CD213a2	CD215 (IL-15Rα)	CD218a (IL-18Rα)

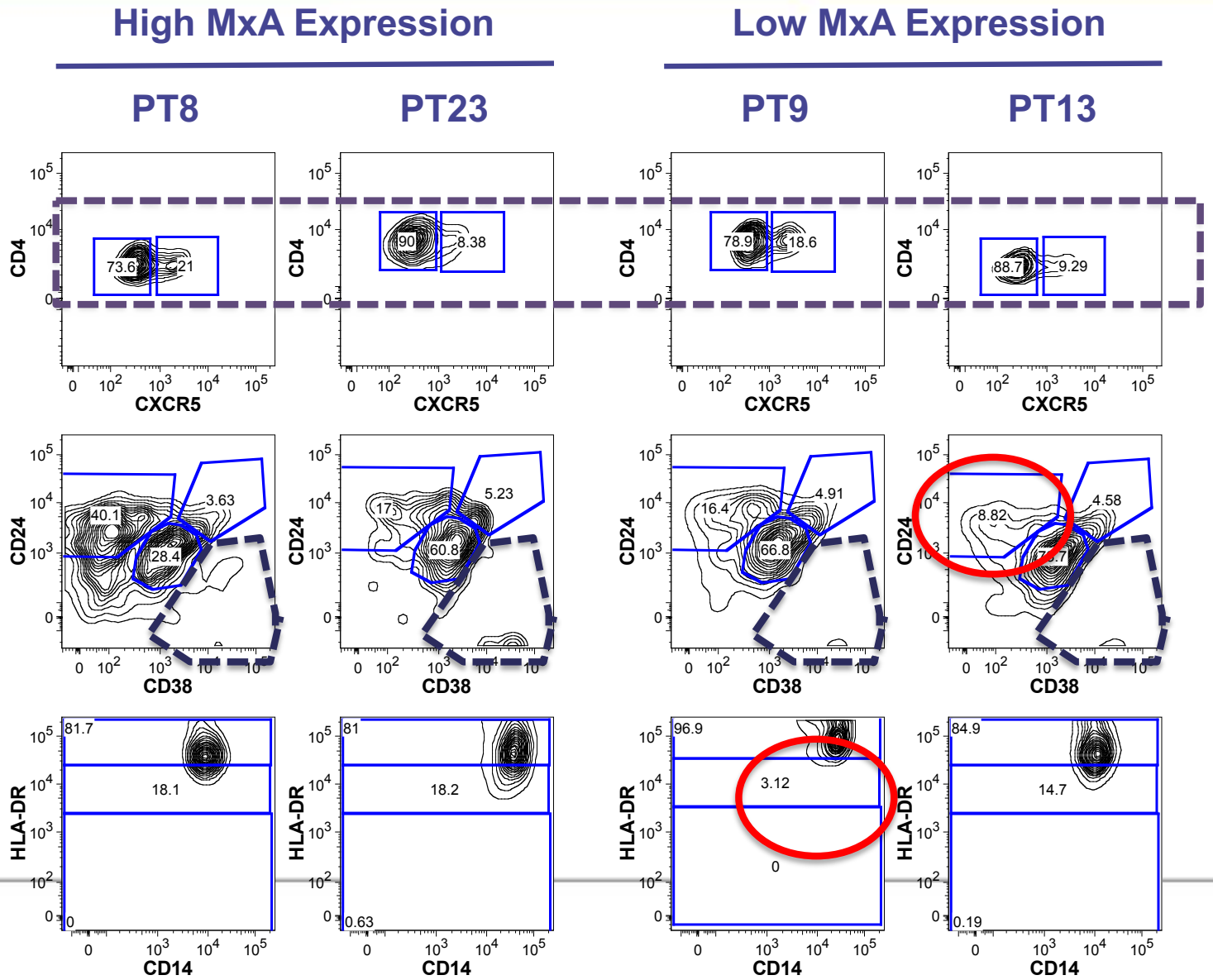
Plate 4

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Integrin α9β1	Integrin β5	Integrin β7	Jagged 2	LAP	LT-βR	Mac-2 (Galectin-3)	MAIR-II	MICA/MICB	MSC (W3D5)	MSC (W5C5)
B	MSC (W7C6)	MSC and NPC (W4A5)	MSCA-1 (MSC)	NKp80	Notch 1	Notch 2	Notch 3	Notch 4	NPC (57D2)	Podoplanin	Pre-BCR	PSMA
C	Siglec-10	Siglec-8	Siglec-9	SSEA-1	SSEA-3	SSEA-4	SSEA-5	TCR γ/δ	TCR Vβ13.2	TCR Vβ23	TCR Vβ8	TCR Vβ9
D	TCR Vδ2	TCR Vγ9	TCR Vα24-Jα18	TCR Vα7.2	TCR α/β	Tim-1	Tim-3	Tim-4	TLT-2	TRA-1-60-R	TRA-1-81	TSLPR (TSLP-R)
E	Mo IgG1, κ ITCL	Mo IgG2a, κ ITCL	Mo IgG2b, κ ITCL	Mo IgG3, κ ITCL	Mo IgM, κ ITCL	Rat IgG1, κ ITCL	Rat IgG2a, κ ITCL	Rat IgG2b, κ ITCL	Rat IgM, κ ITCL	AH IgG, ITCL	Blank	Blank
F	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
G	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

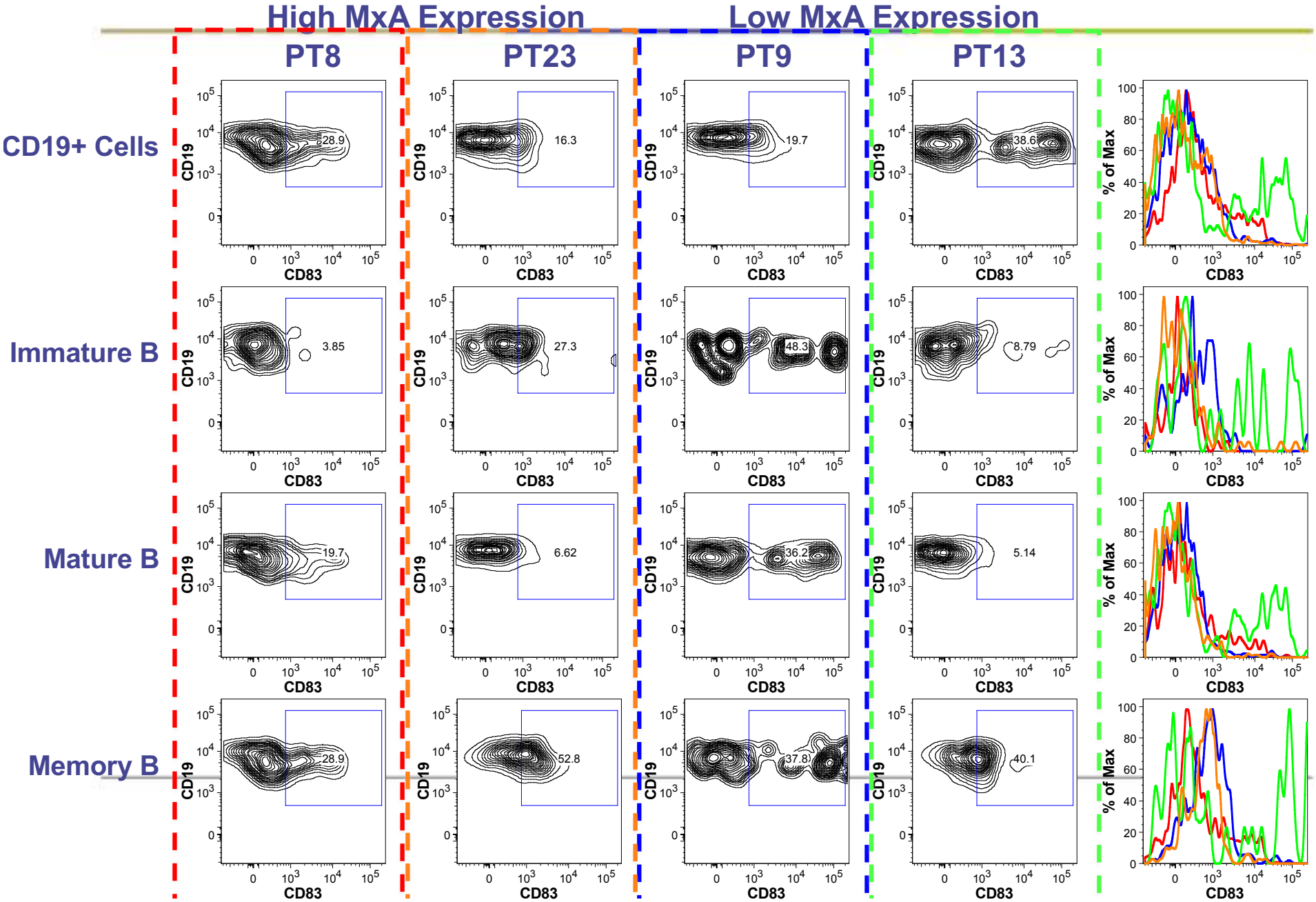
Investigate frequency of different cell populations as well as immune ‘signature’ of specific subpopulations



Preliminary Data



B cells Activation Markers



Conclusions

- Performing LegendScreen analysis on ADA+ and ADA- samples from patients with MS, RA and SLE, IBD cohort to follow
- Developing strategies to analyse the data – advanced statistical help
- Develop custom phenotyping panels for screening the prospective cohorts
- Identified markers analysed further for biological significance

Acknowledgements

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BioLegend

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