Barcoding of live human PBMC for multiplexed mass cytometry

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- supplemenetary material: three figures and one table -

Figure S1

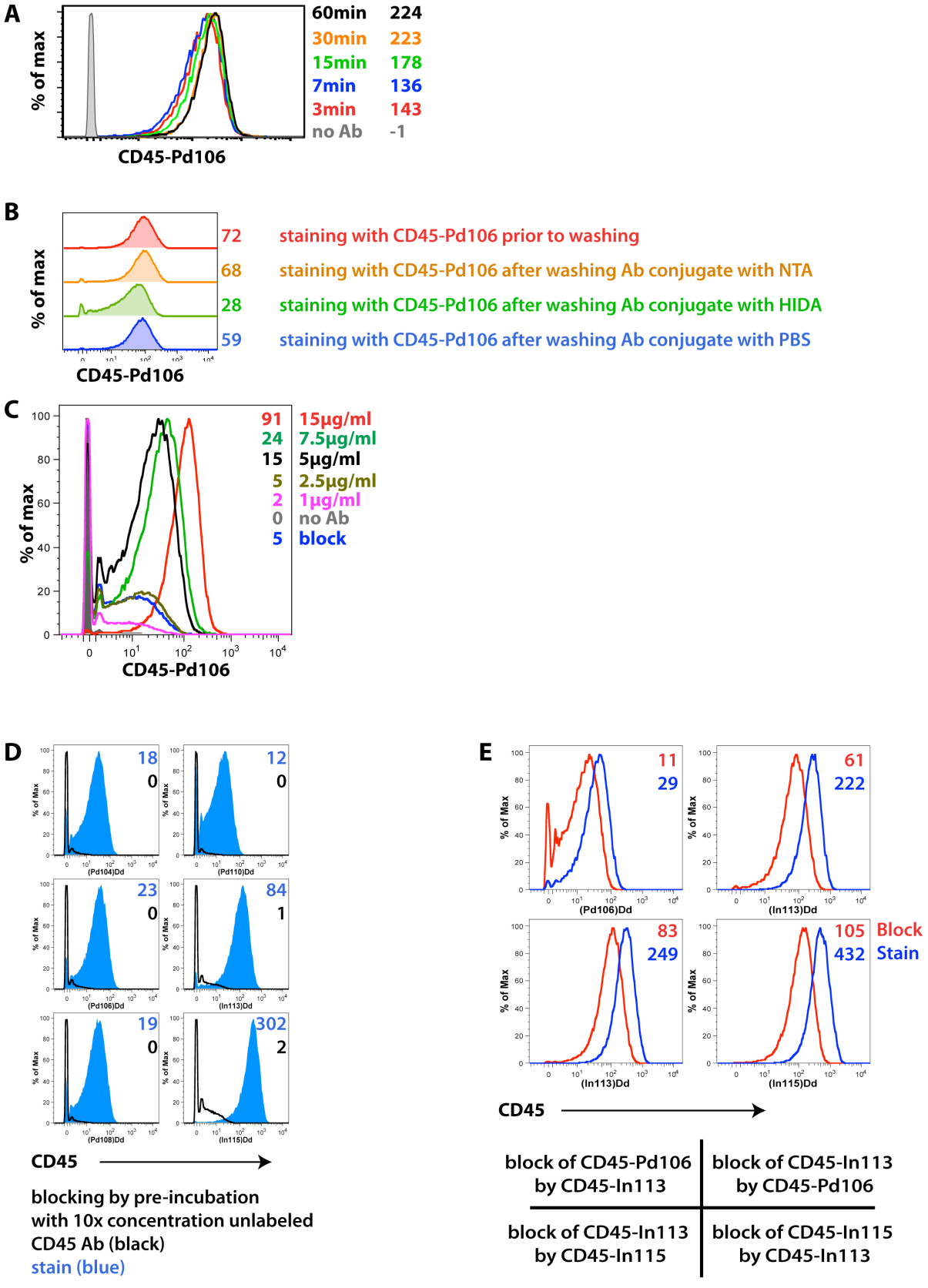
A
% of max
CD45-Pd106
60min 224
30min 223
15min 178
7min 136
3min 143
no Ab -1
B
% of max
CD45-Pd106
72 staining with CD45-Pd106 prior to washing
68 staining with CD45-Pd106 after washing Ab conjugate with NTA
28 staining with CD45-Pd106 after washing Ab conjugate with HIDA
59 staining with CD45-Pd106 after washing Ab conjugate with PBS
C
% of max
CD45-Pd106
91 15µg/ml
24 7.5µg/ml
15 5µg/ml
5 2.5µg/ml
2 1µg/ml
0 no Ab
5 block
D
18 0
12 0
23 0
84 1
19 0
302 2
(Pd104)Dd
(Pd110)Dd
(Pd106)Dd
(In113)Dd
(Pd108)Dd
(In115)Dd
CD45
blocking by pre-incubation with 10x concentration unlabeled CD45 Ab (black)
stain (blue)
E
11 29
61 222
83 249
105 432
Block
Stain
(Pd106)Dd
(In113)Dd
(In115)Dd
CD45
block of CD45-Pd106 by CD45-In113
block of CD45-In113 by CD45-Pd106
block of CD45-In113 by CD45-In115
block of CD45-In115 by CD45-In113

Figure S1. Validation of the CD45-Pd conjugates

(A) PBMC were stained with CD45-Pd106 conjugates that underwent differing conjugation reaction times as indicated. (B) PBMC were stained with CD45-Pd106 that was either left untreated, or was washed three times with counter chelators NTA or HIDA, or with PBS. Pd106 signal intensities were similar confirming high affinity binding/chelation of Pd to the Ab. (C) PBMC were stained with different concentrations of CD45-Pd106. Resulting Pd106 signal intensities were concentration dependent. In histograms showing 2.5µg / mL, 1 µg / mL, "block" and "no Ab", cells of the left histogram leg merge into the peak representing cells with no Pd106 signal, between -1 and 0. Thus, the signal distribution is non-Gaussian in these histograms. (D) PBMC were stained with CD45-Pd104, CD45-Pd106, CD45-Pd108, CD45-Pd110, CD45-In113, or CD45-In115 (all 5 µg/mL), with or without prior incubation with 50 µg/mL unlabeled CD45 Ab. Pre-incubation with excess unlabeled CD45 Ab resulted in the inhibition of the later staining with labeled Ab. (E) PBMC were sequentially stained with CD45-Pd106, CD45-In113, or CD45-In115 (all 5 µg/mL) in the indicated combinations. In- and Pd-labeled CD45 Ab showed similar capabilities of blocking secondary CD45 staining or of being blocked by pre-incubation with differently labeled CD45 Ab. Numbers in A-E indicate geometric mean signal intensities.

Figure S2

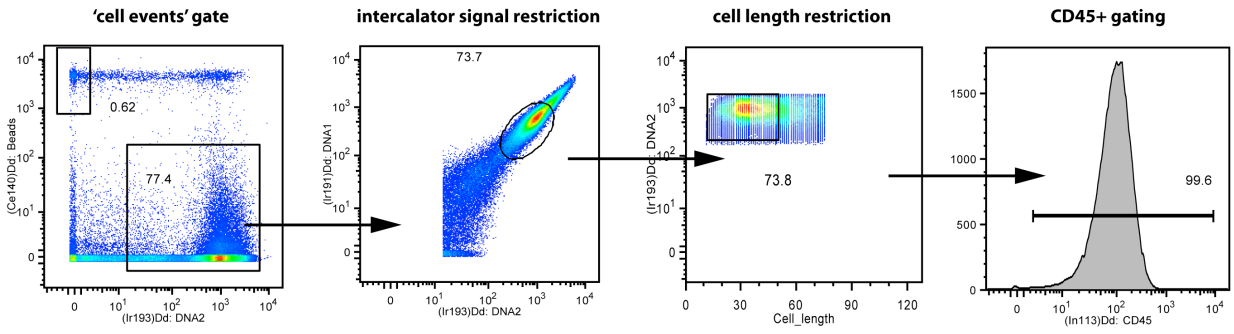

Figure S2. Gating strategy and selection for $CD45^{+}$ cells in individually-processed control PBMC samples

Gating strategy based on DNA staining and 'cell length' parameter as well as CD45-In113 staining.

Figure S3

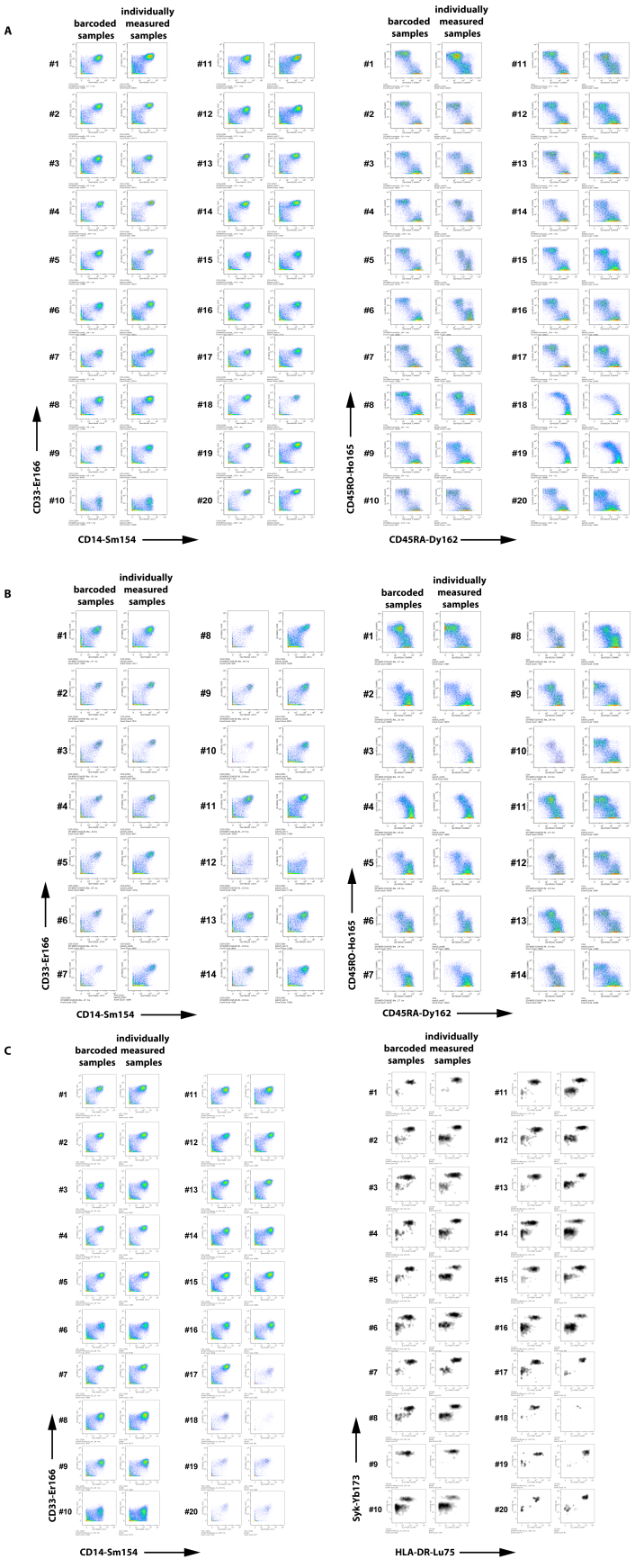
A
barcoded samples
individually measured samples
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
#13
#14
#15
#16
#17
#18
#19
#20
CD33-Er166
CD14-Sm154
CD45RO-Ho165
CD45RA-Dy162
B
barcoded samples
individually measured samples
CD33-Er166
CD14-Sm154
CD45RO-Ho165
CD45RA-Dy162
C
barcoded samples
individually measured samples
CD33-Er166
CD14-Sm154
Syk-Yb173
HLA-DR-Lu75

Figure S3. Reproduction of distinctive features of individual samples from barcoded and deconvoluted sample data.

Series of biaxial dotplots comparing barcoded and individually measured sample from Experiment 1 (A), Experiment 2 (B) and Experiment 3 (C). Biaxial dotplots depicting CD33 vs. CD14 expression are shown where monocytes appear as a separate $CD14^{+}CD33^{+}$ population. The parent population was gated for $CD20^{-}CD3^{-}$ cells events. Note that monocytes in two samples (left panels in A: #10; B: #12, and C: #10) show low or no CD33 expression in both the individually measured as well as in the barcoded sample data. Such $CD14^{+}CD33^{low/-}$ monocytes are not observed in any other barcoded sample, thus confirming the retrieval of the barcoded and only the barcoded cells by the deconvolution strategy. Similar correlations are observed for CD45RA vs. CD45RO staining patterns exhibited by $CD3^{+}CD20^{-}CD4^{+}$ T cells (right panels in A and B) and for Syk vs. HLA-DR staining pattern exhibited by an extremely rare population of $CD3^{-}CD20^{-}CD14^{-}CD33^{-}CD123^{+}$ cells (right panel in C). Syk^{high}HLA-DR^{high} cells represent plasmacytoid dendritic cells while Syk^{low}HLA-DR^{-} events represent basophils.

Table SI. Antibodies used in this study**

antibody target	clone	label	conjugation	source
CD9	HI9a	Pr141	in house	Biolegend
CD19	HIB19	Nd142	in house	Biolegend
CD4	SK3	Nd143	in house	Biolegend
CD8	SK1	Nd144	in house	Biolegend
IgD	IA6-2	Nd146	in house	Biolegend
CD85j	292319	Sm147	in house	R & D Systems
IgA	polyclonal	Nd148	Fluidigm	Fluidigm
CD16	3G8	Sm149	in house	Biolegend
CD3	UCHT1	Nd150	in house	BD Biosciences
CD27	L128	Sm152	in house	BD Biosciences
CD62L	DREG-56	Eu153	Fluidigm	Fluidigm
CD14	M5E2	Sm145	in house	Biolegend
CD32	FLI8.26	Gd155	in house	BD Biosciences
CD94	HP-3D9	Gd156	in house	BD Biosciences
CD11c	Bu15	Tb159	Fluidigm	Fluidigm
CCR7	150503	Gd160	in house	R & D Systems
CD45RA	HI100	Dy162	in house	Biolegend
BDCA3	AD5-14H12	Dy163	in house	Miltenyi Biotec
CD20	2H7	Dy164	in house	Biolegend
CD45RO	UCHL1	Ho165	Fluidigm	Fluidigm
CD33	P67.6	Er166	in house	Santa Cruz
CD28	L293	Er167	in house	BD Biosciences
CD24	ML5	Er168	in house	Biolegend
CD25	2A3	Tm169	Fluidigm	Fluidigm
CD161	DX12	Er170	in house	BD Biosciences
TCRgd	B1	Yb171	in house	Biolegend
CD38	HIT2	Yb172	Fluidigm	Fluidigm
cyt Syk	4D10	Yb173	in house	BD Biosciences
HLADR	G46-6	Lu175	in house	BD Biosciences
CD56	HCD56	Yb176	in house	Biolegend
CD43	84-3C1	Nd150	Fluidigm	Fluidigm
CD123	6H6	Eu151	Fluidigm	Fluidigm
CD86	IT2.2	Gd157	in house	Biolegend
CD3	UCHT1	Er170	Fluidigm	Fluidigm
CD66b	CD66a-B1.1	Yb171	Fluidigm	Fluidigm
mouse CD45	30-F11	La139	in house	Biolegend
CD45	Hi30	Pd104	in house	Biolegend
CD45	Hi30	Pd106	in house	Biolegend
CD45	Hi30	Pd108	in house	Biolegend
CD45	Hi30	Pd110	in house	Biolegend

CD45	Hi30	In113	in house	Biolegend
CD45	Hi30	In115	in house	Biolegend

**Antibodies labeled in-house were conjugated using MAXPAR® conjugation kits (Fluidigm, Sunnyvale, CA) according to a protocol based on the manufacturer's instructions, or as described in "Materials and Methods".