Supplementary Table 1

Subject	*Bone lesions*	*Calcium (mg/dl)*	*Creatinine (mg/dl)*	*Hemoglobin (g/dl)*
3	No	9.0	0.8	10.5
4	No	9.0	0.7	13.8
6	No	9.6	0.9	11.4
7	Yes	8.9	0.7	12.9
8	Yes	10.1	1.3	12.2
10	Yes	10.0	1.1	9.9
11	No	9.5	0.8	9.0
18	No	8.8	1.3	12.9
19	No	8.6	0.7	10.8
20	Yes	8.9	1.9	9.4
21	Yes	8.7	1.6	12.1
25	No	8.8	1.4	11.1
32	Yes	11.1	0.9	11.1

Supplementary Table 1: Supplementary subject data

Additional clinical and laboratory data for all 13 MM subjects included in the study

Supplementary Table 2

Reagent	*Final conc.*	*Manufacturer*	*Medium/Buffer*
RPMI1640		Gibco Life Technologies	Cell culture medium
FBS	10%	Sigma Aldrich	Cell culture medium
Sodium pyruvate	1mM	Gibco Life Technologies	Cell culture medium
MEM non-essential amino acids	1x	Gibco Life Technologies	Cell culture medium
Penicillin	5U/ml	Gibco Life Technologies	Cell culture medium
Streptomycin	5ug/ml	Gibco Life Technologies	Cell culture medium
L-glutamine	1.46mg/ml	Gibco Life Technologies	Cell culture medium
10x PBS	1x	Ambion Life Technologies	CyFACS
BSA	0.5%	Sigma Aldrich	CyFACS
Sodium azide	0.02%	VWR	CyFACS
EDTA	2mM	Rockland	CyFACS

Supplementary Table 2: CyFACS buffer and cell culture medium recipes

All buffers and cell culture medium were filtered through a 0.2 µm filter.

Supplementary Table 3

Antibody	*Staining*	*Clone*	*Manufacturer*	*Label*	*Final conc.(µg/ml)*
IgM	S	G20-127	BD	139 La	8
CD3	S	UCHT1	Invitrogen	Qdot 605	2µl
CD3	S	UCHT1	BioLegend	141 Pr	2
CD33	S	P67.6	Santa Cruz	142 Nd	2
CD4	S	SK3	BioLegend	143 Nd	4
CD57	S	HCD57	BioLegend	145 Nd	1
CD8	S	SK1	BioLegend	146 Nd	2
CD45	S	HI30	BioLegend	147 Sm	1.2
IgD	S	IA6-2	BD	149 Sm	8
CD16	S	B73.1	affymetrix	150 Nd	12
CD19	S	HIB19	BioLegend	151 Eu	4
CD11c	S	Bly-6	BD	152 Sm	8
CD24	S	ML5	BioLegend	153 Eu	8
CD27	S	LG.7F9	affymetrix	154 Sm	8
CD10	S	HI10a	BioLegend	155 Gd	10
CD1c	S	L161	BioLegend	156 Gd	6
CD14	S	M5E2	BioLegend	157 Gd	8
CCR7	S	150503	R&D	158 Gd	8
CD7	S	M-T701	BD	159 Tb	2
CD28	S	CD28.2	BD	160 Gd	12
CD5	S	UCHT2	BioLegend	161 Dy	8
CD38	S	HIT2	BioLegend	165 Ho	8
CD45RA	S	HI100	BioLegend	166 Er	8
CD123	S	9F5	BD	167 Er	2
CD66	S	B1.1/CD66	BD	168 Er	2
CD45RO	S	UCHL1	BioLegend	169 Tm	8
CD56	S	NCAM16.2	BD	170 Er	12
HLA-DR	S	L243	BioLegend	171 Yb	4
TCR $\gamma\delta$	S	5A6.E9	life	PE	2
Anti PE	S	PE0001	BioLegend	172 Yb	6
CD25	S	M-A251	BD	173 Yb	12
TCR $\alpha\beta$	S	IP26	BioLegend	174 Yb	12
CD127	S	A019D5	BioLegend	176 Yb	12
IFN-γ	I	ebio4S.B3	affymetrix	148 Nd	2
TNF-α	I	MAb11	BioLegend	162 Dy	8
IL4	I	8D4-8	BD	163 Dy	8
IL17A	I	BL23	BioLegend	164 Dy	8
IFN-α	I	LT27:295	Miltenyi	175 Lu	8
CD20	I	H1	BD	144 Nd	8

Supplementary Table 3: Antibodies used for CyTOF staining

S: surface; I: intracellular; PE: Phycoerythrin

Supplementary Table 4

Reagent	*Fluorchrome*	*Clone*	*Manufacturer*	*Experiment*
CD24	BV421	ML5	BioLegend	Phosphoflow
CD38	PE-Cy7	HB8	BD	Phosphoflow
CD27	BV605	L128	BD	Phosphoflow
CD20	PerCP-Cy5.5	H1	BD	Phosphoflow
p-SYK	AF647	17A/P-ZAP70	BD	Phosphoflow
p-p38	PE	36/p38	BD	Phosphoflow
ERK1/2		197G2	CST	Phosphoflow
Goat anti rabbit	AF488		Invitrogen	Phosphoflow
CD20	PerCP-Cy5.5	2H7	BioLegend	Singe cell sort
CD24	APC	ML5	BioLegend	Singe cell sort
CD38	PE	HIT2	BioLegend	Singe cell sort
IgD	BV421	IA6-2	BioLegend	Singe cell sort
IgM	BV510	MHM-88	BioLegend	Singe cell sort
CD19	BV650	HIB19	BioLegend	Singe cell sort
CD14	APC-Cy7	M5E2	BioLegend	Singe cell sort
CD27	PE-Cy7	LG.7F9	eBiosciences	Singe cell sort
IgA	FITC	IS11-8E10	Miltenyi	Singe cell sort
NHS ester	DyLight800		Fisher Scientific	Singe cell sort
Zombie NIR fixable viability kit	NIR		BioLegend	Phenotype analysis

Supplementary Table 4: Antibodies/Reagents used for FACS staining

Supplementary Table 5

1 B/unstim
2 B_CD20+CD38+/unstim
3 B_CD20+CD27+/unstim
4 B_CD38+IgD+/unstim
5 B_CD38-IgD+/unstim
6 B_CD20+IgM+/unstim
7 B_memory/unstim
8 B_memory_CD20+CD27+/unstim
9 B_memory_CD20+CD10+/unstim
10 B_memory_CD20+CD5+/unstim
11 B_naive/unstim
12 B_naive_CD20+CD27+/unstim
13 B_naive_CD20+CD10+/unstim
14 B_naive_CD20+CD5+/unstim
15 B_transitional/unstim
16 B_transitional_CD20+CD27+/unstim
17 B_transitional_CD20+CD5+/unstim
18 B_transitional_CD20+CD10+/unstim
19 plasmablast/unstim
20 NK/unstim
21 NK_CD56++CD16-/unstim
22 NK_CD56+CD16+/unstim
23 NK_56+16-/unstim
24 monocytes/unstim
25 monocytes_CD16-CD14+/unstim
26 monocytes_CD16+CD14-/unstim
27 myeloid_DC/unstim
28 mDCs/unstim
29 pDC/unstim
30 Tab_percent/unstim
31 Tab_CD4+/unstim
32 Tab_memory_CD4+/unstim
33 Tab_memory_CD4+CCR7+/unstim
34 Tab_memory_CD4+CD28+/unstim
35 Tab_memory_CD4+CD57+/unstim
36 Tab_naive_CD4+/unstim
37 Tab_naive_CD4+CCR7+/unstim
38 Tab_naive_CD4+CD28+/unstim
39 Tab_naive_CD4+CD57+/unstim
40 Tab_CD4+CD45RO-CD28+/unstim
41 Tab_CD4+CD45RO+CD28+/unstim
42 Tab_CD4+CD45RO+CD28-/unstim
43 Tab_CD4+CD28-CD45RO-/unstim
44 Tab_CD4+CD38-HLA-DR+/unstim
45 Tab_CD4+CD38+HLA-DR+/unstim
46 Tab_CD4+CD45RO-CD38+/unstim
47 Tab_CD4+CD45RO+CD38+/unstim
48 Treg/unstim
49 Treg_CD45RO+/unstim
50 Tab_CD4+CD8+/unstim
51 Tab_CD8+/unstim
52 Tab_CD8+CD38-HLA-DR+/unstim
53 Tab_CD8+CD38+HLA-DR+/unstim
54 Tab_memory_CD8+/unstim
55 Tab_memory_CD8+CD57+/unstim
56 Tab_memory_CD8+CCR7+/unstim
57 Tab_memory_CD8+CD28+/unstim
58 Tab_naive_CD8+/unstim
59 Tab_naive_CD8+CCR7+/unstim
60 Tab_naive_CD8+CD28+/unstim
61 Tab_naive_CD8+CD57+/unstim
62 Tab_CD8+CD45RA-CD28+/unstim
63 Tab_CD8+CD45RA+CD28+/unstim
64 Tab_CD8+CD45RA+CD28-/unstim
65 Tab_CD8+CD45RA-CD28-/unstim
66 Tab_CD8+CD45RA-CD38+/unstim
67 Tab_CD8+CD45RA+CD38+/unstim
68 Tgd_percent/unstim
69 Tgd_CD27-CD38+/unstim
70 Tgd_CD27+CD38+/unstim
71 Tgd_CD27+CD38-/unstim
72 Tgd_CD27-CD38-/unstim
73 Tgd_CD28-CD38+/unstim
74 Tgd_CD28+CD38+/unstim
75 Tgd_CD28+CD38-/unstim
76 Tgd_CD28-CD38-/unstim
77 Tgd_CD45RA-CD38+/unstim
78 Tgd_CD45RA+CD38+/unstim
79 Tgd_CD45RA+CD38-/unstim
80 Tgd_CD45RA-CD38-/unstim
81 Tgd_CD16-CD38-/unstim
82 Tgd_CD16-CD38+/unstim

83 Tgd_CD16+CD38-/unstim
84 Tgd_CD16+CD38+/unstim
85 Tgd_CD38-HLA-DR-/unstim
86 Tgd_CD38+CD11c-/unstim
87 Tgd_CD38+CD11c+/unstim
88 Tgd_CD38+CD11c+HLA-DR+/unstim
89 Tgd_CD38+HLA-DR-/unstim
90 Tgd_CD38+HLA-DR+/unstim
91 Tgd_CD38-CD11c-/unstim
92 Tgd_CD38-CD11c+/unstim
93 Tgd_CD38-HLA-DR+/unstim
94 Tgd_CD57-CD38-/unstim
95 Tgd_CD57-CD38+/unstim
96 Tgd_CD57+CD38-/unstim
97 Tgd_CD57+CD38+/unstim
98 B/PMA_Iono
99 B_CD20+CD38+/PMA_Iono
100 B_CD20+CD27+/PMA_Iono
101 B_CD20+IFNg+/PMA_Iono
102 B_CD20+IL4+/PMA_Iono
103 B_CD20+TNFa+/PMA_Iono
104 B_CD38+IgD+/PMA_Iono
105 B_CD38-IgD+/PMA_Iono
106 B_CD20+IgM+/PMA_Iono
107 B_memory/PMA_Iono
108 B_memory_CD20+CD27+/PMA_Iono
109 B_memory_TNFa+/PMA_Iono
110 B_memory_CD20+CD10+/PMA_Iono
111 B_memory_CD20+CD5+/PMA_Iono
112 B_naive/PMA_Iono
113 B_naive_CD20+CD27+/PMA_Iono
114 B_naive_TNFa+/PMA_Iono
115 B_naive_CD20+CD10+/PMA_Iono
116 B_naive_CD20+CD5+/PMA_Iono
117 B_transitional/PMA_Iono
118 B_transitional_CD20+CD27+/PMA_Iono
119 B_transitional_TNFa+/PMA_Iono
120 B_transitional_CD20+CD5+/PMA_Iono
121 B_transitional_CD20+CD10+/PMA_Iono
122 plasmablast/PMA_Iono
123 NK/PMA_Iono
124 NK_CD56++CD16-/PMA_Iono
125 NK_CD56+CD16+/PMA_Iono
126 NK_IFNg+/PMA_Iono
127 NK_IL4+/PMA_Iono
128 NK_TNF+/PMA_Iono
129 NK_56+16-/PMA_Iono
130 monocytes/PMA_Iono
131 monocytes_CD16-CD14+/PMA_Iono
132 monocytes_16-14+TNFa+/PMA_Iono
133 monocytes_CD16+CD14-/PMA_Iono
134 myeloid_DC/PMA_Iono
135 mDCs/PMA_Iono
136 mDC_TNFa+/PMA_Iono
137 pDC/PMA_Iono
138 pDC_TNFa+/PMA_Iono
139 Tab_percent/PMA_Iono
140 Tab_CD4+/PMA_Iono
141 Tab_CD4+CD8+/PMA_Iono
142 Tab_CD8-IFNg+/PMA_Iono
143 Tab_CD8-IL4+/PMA_Iono
144 Tab_CD8-IL17+/PMA_Iono
145 Tab_CD8-TNFa+/PMA_Iono
146 Tab_CD8+/PMA_Iono
147 Tab_CD8+IFNg+/PMA_Iono
148 Tab_CD8+IL4+/PMA_Iono
149 Tab_CD8+IL17+/PMA_Iono
150 Tab_CD8+TNFa+/PMA_Iono
151 Tgd_percent/PMA_Iono
152 Tgd_CD38-IFNg+/PMA_Iono
153 Tgd_CD38-IL4+/PMA_Iono
154 Tgd_CD38-IL17A+/PMA_Iono
155 Tgd_CD38-TNFa+/PMA_Iono
156 Tgd_CD38+IFNg+/PMA_Iono
157 Tgd_CD38+IL17A+/PMA_Iono
158 Tgd_CD38+TNFa+/PMA_Iono
159 B/R848
160 B_CD20+CD38+/R848
161 B_CD20+CD27+/R848
162 B_CD20+IFNg+/R848
163 B_CD20+IL4+/R848
164 B_CD20+TNFa+/R848
165 B_CD38+IgD+/R848
166 B_CD38-IgD+/R848
167 B_CD20+IgM+/R848
168 B_memory/R848

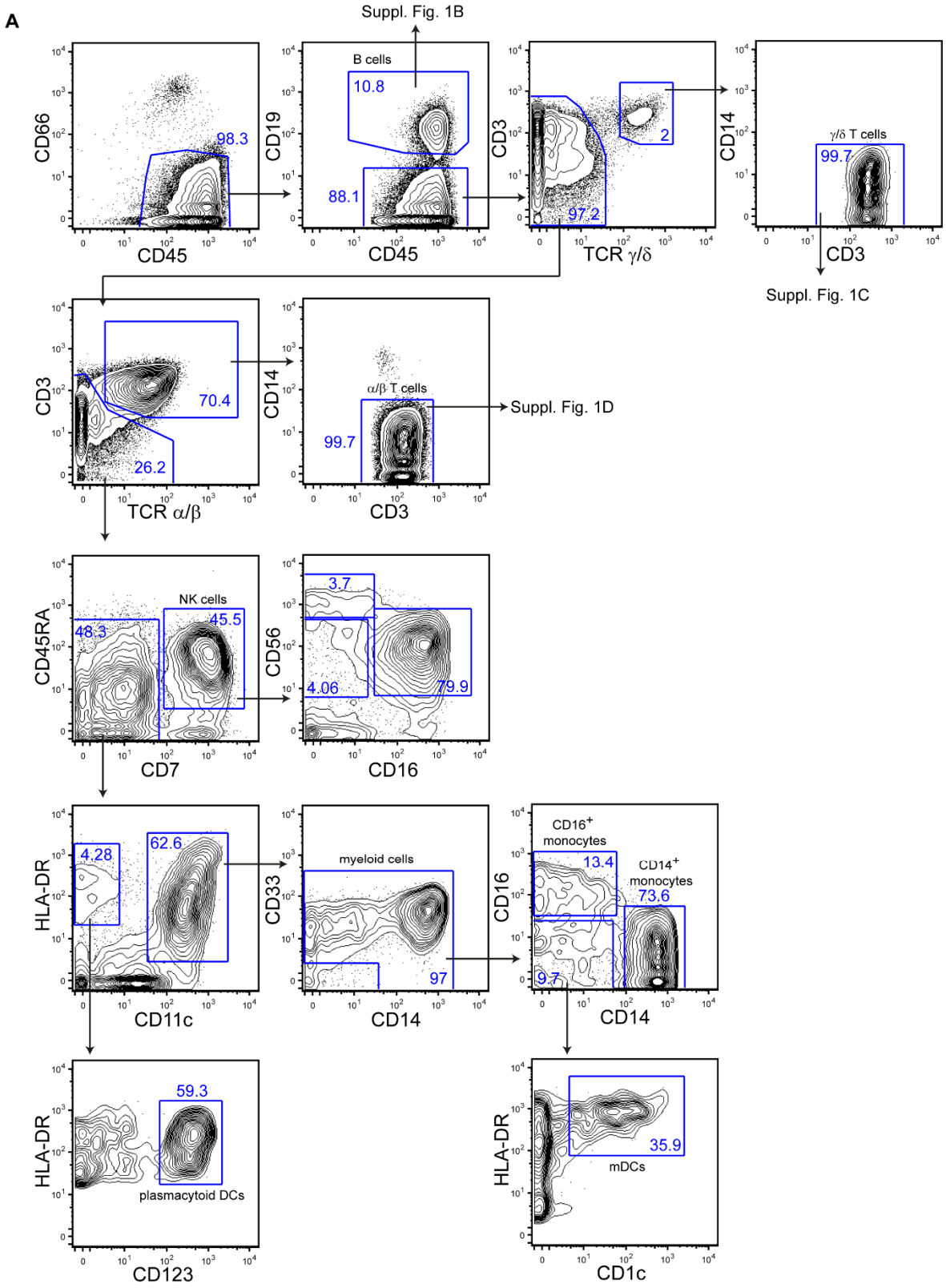
169 B_memory_CD20+CD27+/R848
170 B_memory_TNFa+/R848
171 B_memory_CD20+CD10+/R848
172 B_memory_CD20+CD5+/R848
173 B_naive/R848
174 B_naive_CD20+CD27+/R848
175 B_naive_TNFa+/R848
176 B_naive_CD20+CD10+/R848
177 B_naive_CD20+CD5+/R848
178 B_transitional/R848
179 B_transitional_CD20+CD27+/R848
180 B_transitional_TNFa+/R848
181 B_transitional_CD20+CD5+/R848
182 B_transitional_CD20+CD10+/R848
183 plasmablast/R848
184 NK/R848
185 NK_CD56++CD16-/R848
186 NK_CD56+CD16+/R848
187 NK_56+16-/R848
188 monocytes/R848
189 monocytes_CD16-CD14+/R848
190 monocytes_16-14+TNFa+/R848
191 monocytes_CD16+CD14-/R848
192 myeloid_DC/R848
193 mDCs/R848
194 mDC_IL4+/R848
195 mDC_TNFa+/R848
196 pDC/R848
197 pDC_IFNa+/R848
198 pDC_TNFa+/R848
199 Tab_percent/R848
200 Tab_CD4+/R848
201 Tab_CD4+CD8+/R848
202 Tab_CD8+/R848
203 Tgd_percent/R848
204 B_CD20+CD38+/CpG_Dotap
205 B_CD20+CD27+/CpG_Dotap
206 B_CD20+IFNg+/CpG_Dotap
207 B_CD20+IL4+/CpG_Dotap
208 B_CD20+TNFa+/CpG_Dotap
209 B_CD38+IgD+/CpG_Dotap
210 B_CD38-IgD+/CpG_Dotap
211 B_CD20+IgM+/CpG_Dotap
212 B_memory/CpG_Dotap
213 B_memory_CD20+CD27+/CpG_Dotap
214 B_memory_TNFa+/CpG_Dotap
215 B_memory_CD20+CD10+/CpG_Dotap
216 B_memory_CD20+CD5+/CpG_Dotap
217 B_naive/CpG_Dotap
218 B_naive_CD20+CD27+/CpG_Dotap
219 B_naive_TNFa+/CpG_Dotap
220 B_naive_CD20+CD10+/CpG_Dotap
221 B_naive_CD20+CD5+/CpG_Dotap
222 B_transitional/CpG_Dotap
223 B_transitional_CD20+CD27+/CpG_Dotap
224 B_transitional_TNFa+/CpG_Dotap
225 B_transitional_CD20+CD5+/CpG_Dotap
226 B_transitional_CD20+CD10+/CpG_Dotap
227 plasmablast/CpG_Dotap
228 NK/CpG_Dotap
229 NK_CD56++CD16-/CpG_Dotap
230 NK_CD56+CD16+/CpG_Dotap
231 NK_56+16-/CpG_Dotap
232 monocytes/CpG_Dotap
233 monocytes_CD16-CD14+/CpG_Dotap
234 monocytes_CD16+CD14-/CpG_Dotap
235 myeloid_DC/CpG_Dotap
236 mDCs/CpG_Dotap
237 pDC/CpG_Dotap
238 pDC_IFNa+/CpG_Dotap
239 pDC_TNFa+/CpG_Dotap
240 Tab_percent/CpG_Dotap
241 Tab_CD4+/CpG_Dotap
242 Tab_CD4+CD8+/CpG_Dotap
243 Tab_CD8+/CpG_Dotap
244 Tgd_percent/CpG_Dotap

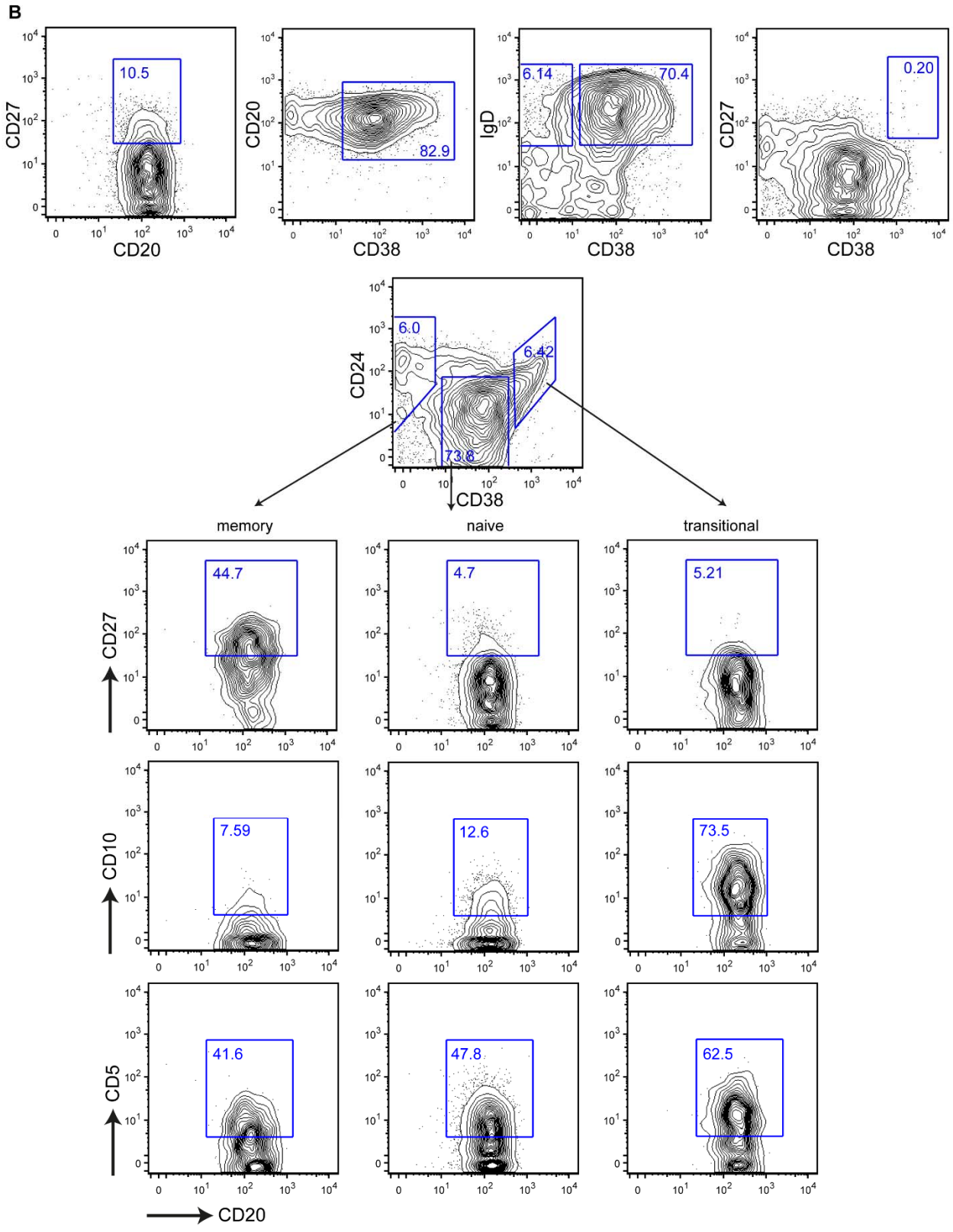
Supplementary Table 5: Two-dimensional CyTOF gates

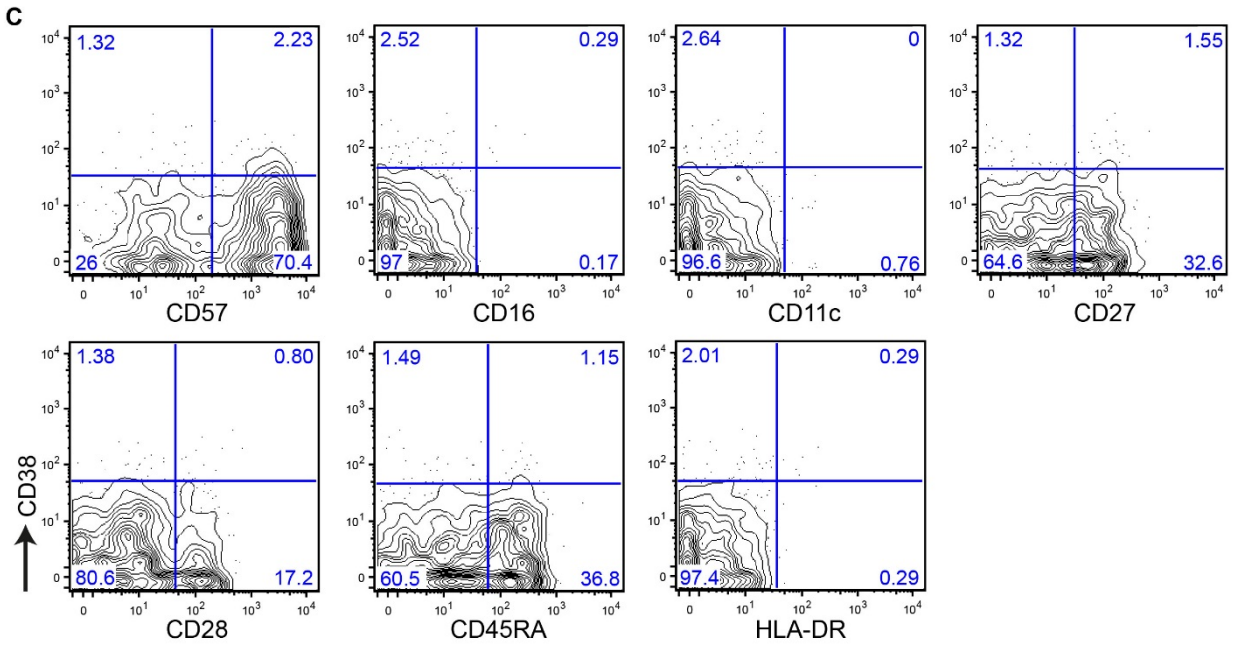
B: $CD66^{-}CD45^{+}CD19^{+}$; plasmablast: $CD66^{-}CD45^{+}CD19^{+}CD38^{hi}CD27^{+}$; NK: $CD66^{-}CD45^{+}CD19^{-}CD3^{-}TCR\gamma/\delta^{-}TCR\alpha/\beta^{-}CD45RA^{+}CD7^{+}$; Tab: $CD66^{-}CD45^{+}CD3^{+}$ $TCR\alpha/\beta^{+}$; monocytes: $CD66^{-}$

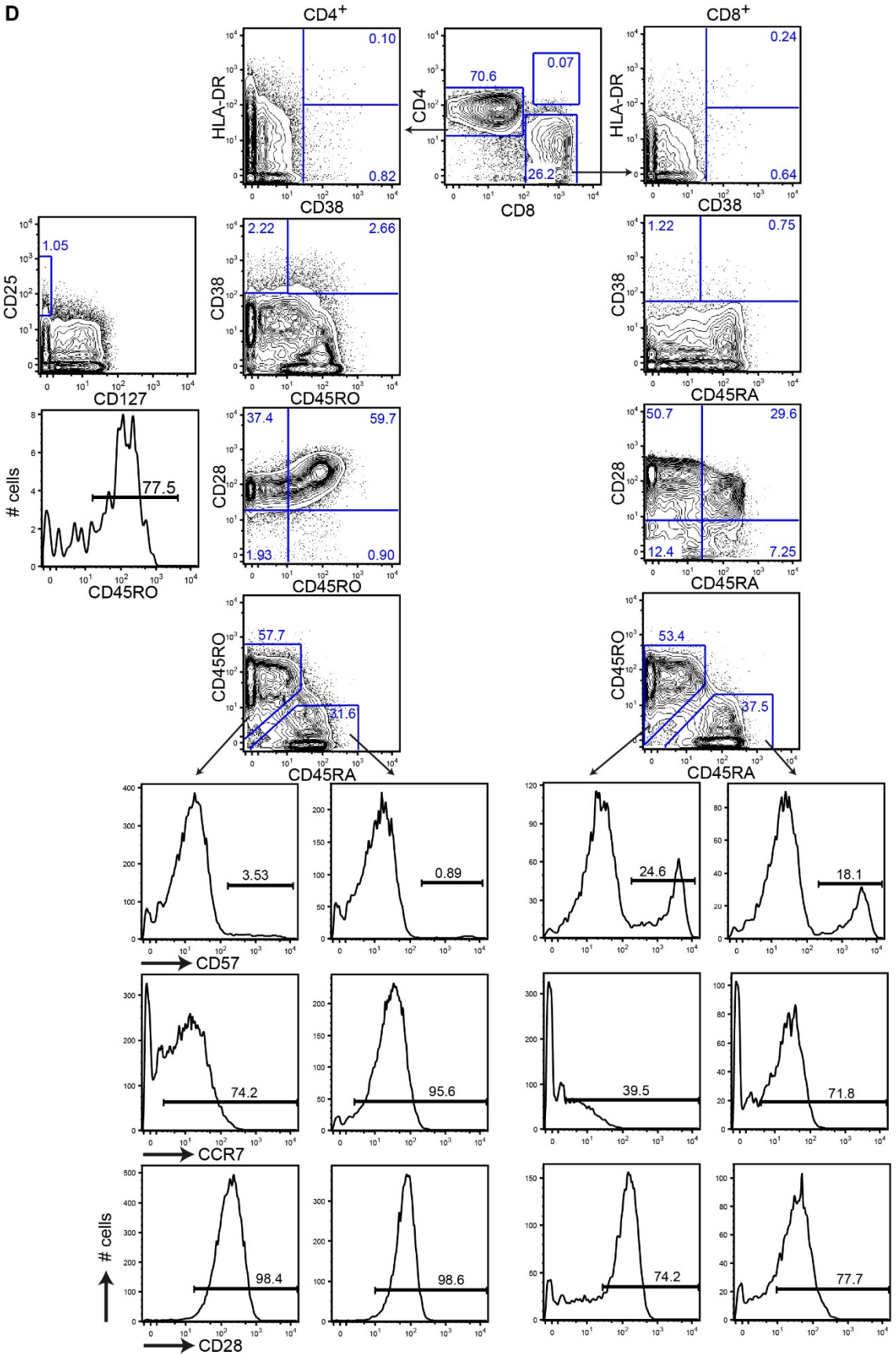
$CD45^+CD19^-CD3^-TCR\gamma/\delta^-TCR\alpha/\beta^-CD45RA^-CD7^-CD11c^+HLA\text{-}DR^+CD33^{+/-}CD14^{+/-}$; myeloid_DC: $CD66^-CD45^+CD19^-CD3^-TCR\gamma/\delta^-TCR\alpha/\beta^-CD45RA^-CD7^-CD11c^+HLA\text{-}DR^+CD33^{+/-}CD14^-CD16^-$; mDCs: $CD66^-CD45^+CD19^-CD3^-TCR\gamma/\delta^-TCR\alpha/\beta^-CD45RA^-CD7^-CD11c^+HLA\text{-}DR^+CD33^{+/-}CD14^-CD16^-CD1c^+$; pDCs: $CD66^-CD45^+CD19^-CD3^-TCR\gamma/\delta^-TCR\alpha/\beta^-CD45RA^-CD7^-CD11c^-HLA\text{-}DR^+CD123^+$; Tab: $CD66^-CD45^+CD19^-CD3^+TCR\gamma/\delta^-TCR\alpha/\beta^+$; Treg: $CD66^-CD45^+CD19^-CD3^+TCR\gamma/\delta^-TCR\alpha/\beta^+CD4^+CD25^{hi}CD127^-$; Tgd: $CD66^-CD45^+CD19^-CD3^+TCR\gamma/\delta^+$. A gating example is shown in Supplementary Figure 1. All gates are pre-gated on DNA^+ maleimide-$DOTA^-$ live events.

Supplementary Fig 1




B
CD27
CD20
10.5
CD20
CD38
82.9
IgD
CD38
6.14
70.4
CD27
CD38
0.20
CD24
CD38
6.0
6.42
73.8
memory
naive
transitional
CD27
44.7
4.7
5.21
CD10
7.59
12.6
73.5
CD5
41.6
47.8
62.5
CD20

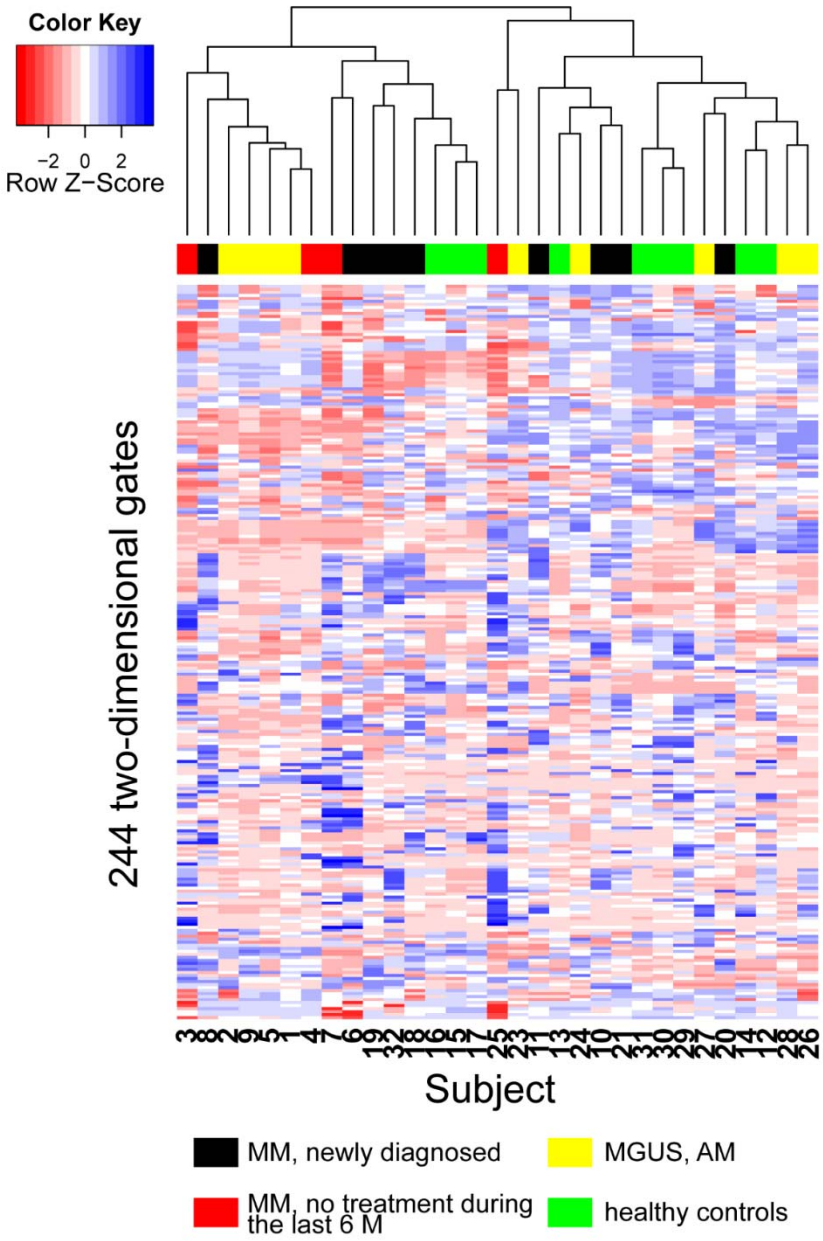

C
1.32
2.23
26
70.4
CD57
2.52
0.29
97
0.17
CD16
2.64
0
96.6
0.76
CD11c
1.32
1.55
64.6
32.6
CD27
1.38
0.80
80.6
17.2
CD28
1.49
1.15
60.5
36.8
CD45RA
2.01
0.29
97.4
0.29
HLA-DR
CD38


D
CD4+
CD8+
HLA-DR
CD38
CD4
CD8
0.10
0.82
70.6
0.07
26.2
0.24
0.64
CD25
CD127
1.05
CD38
CD45RO
2.22
2.66
CD45RA
1.22
0.75
cells
77.5
CD28
37.4
59.7
1.93
0.90
50.7
29.6
12.4
7.25
CD45RO
57.7
31.6
CD45RA
53.4
37.5
3.53
0.89
24.6
18.1
CD57
74.2
95.6
39.5
71.8
CCR7
98.4
98.6
74.2
77.7
CD28

Supplementary Figure 1: Typical two-dimensional identification of PBMC subpopulations

All cells are pre-gated on DNA^+ maleimide-$DOTA^-$ live cells. (A) major PBMC subpopulations, (B) B cell gates, (C) γ/δ T cell gates, and (D) α/β T cell gates. Numbers in the gates indicate frequencies of the corresponding parent populations.

Supplementary Figure 2

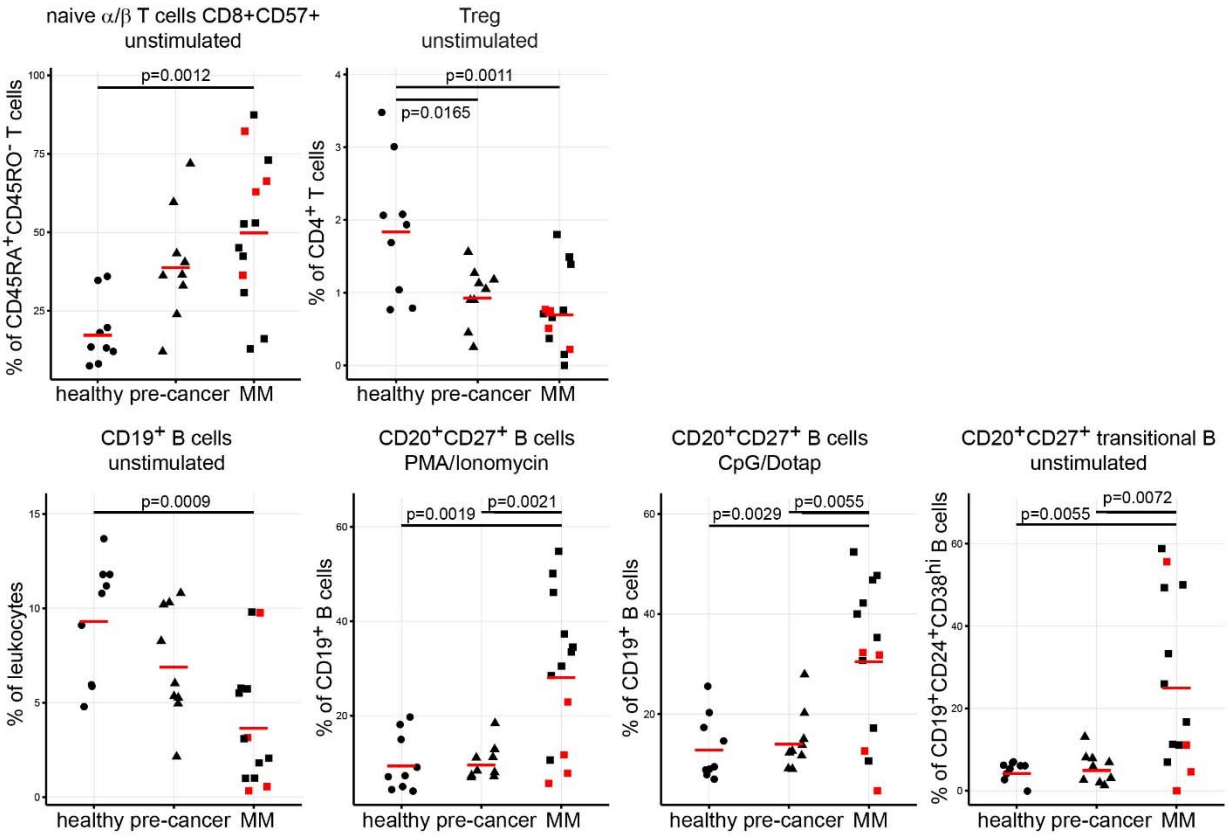


Supplementary Figure 2: Heterogeneity of immune phenotypes in MM, AM, and MGUS patients

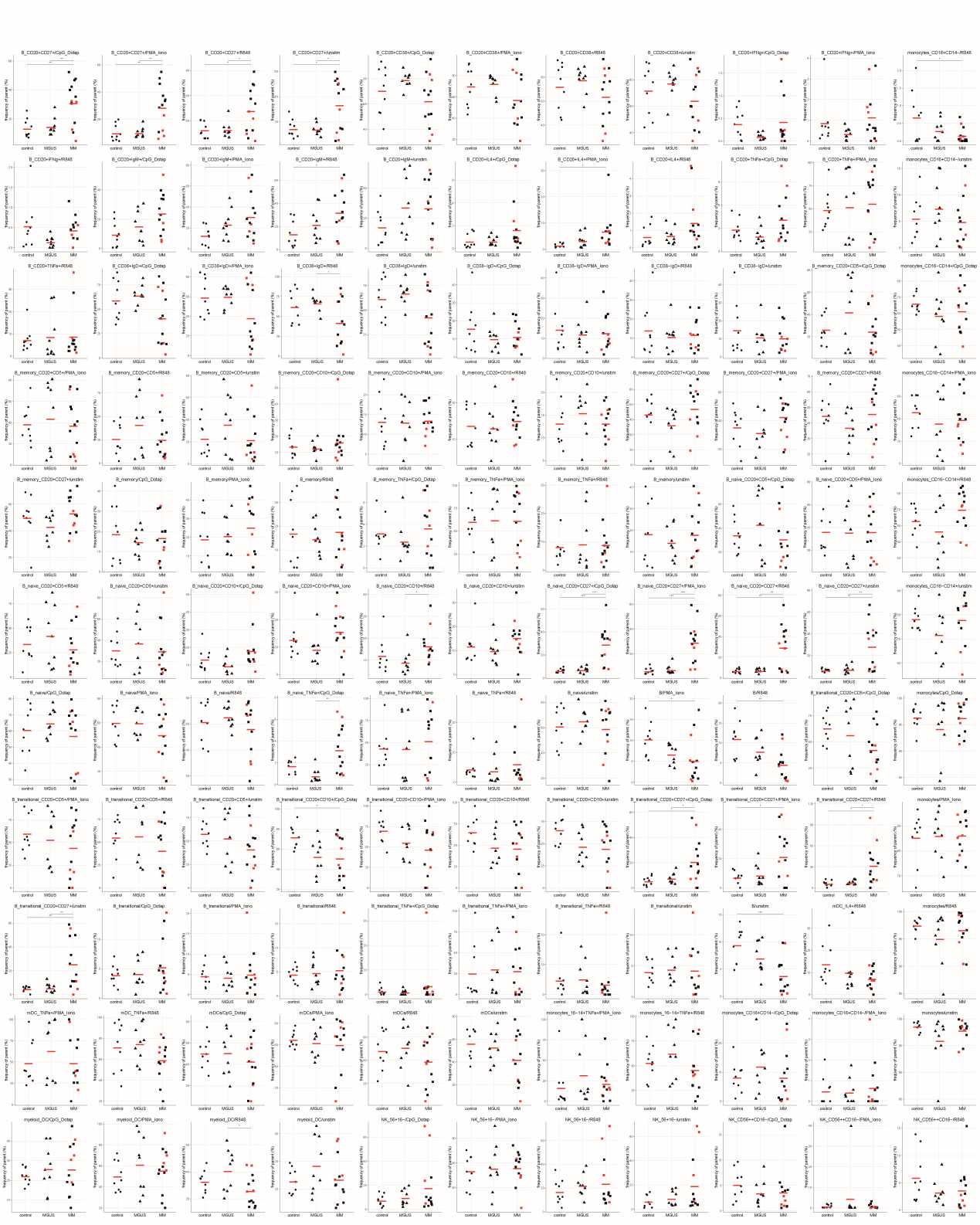
Normalized cell frequencies in 244 CyTOF gates are displayed in lines, samples in columns. Columns were clustered by Euclidian distances between samples based on gated cell frequencies. The subject numbers here correspond to those in Table 1.

Supplementary Figure 3

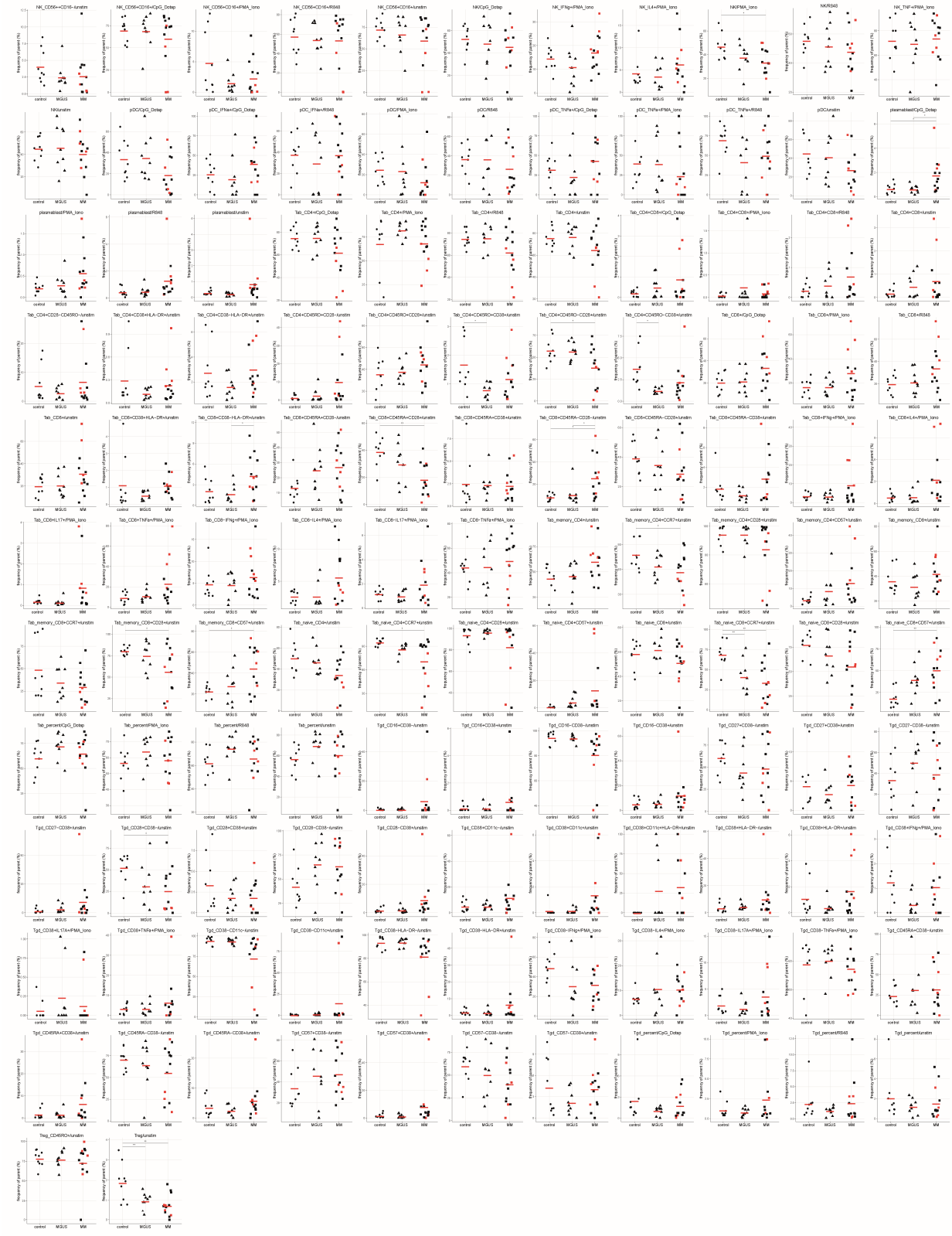
A



Supplementary Figure 3 continued
B



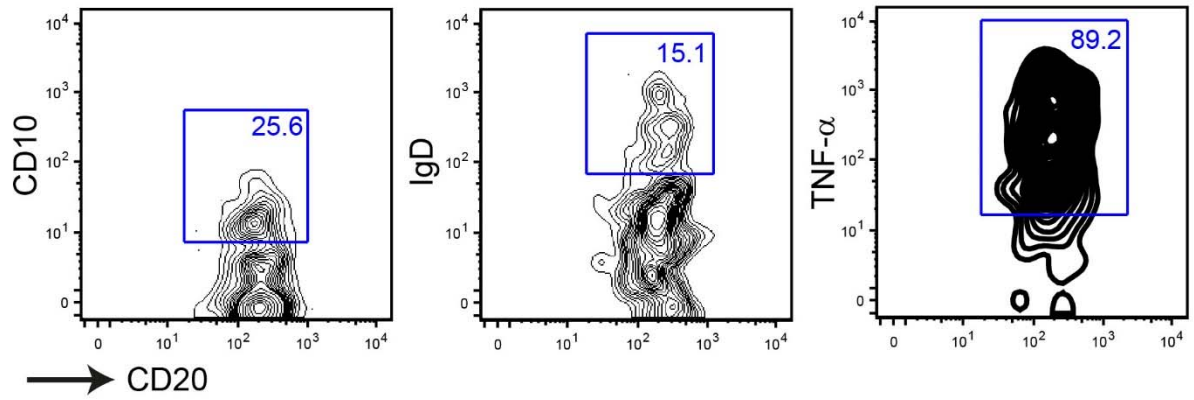
Supplementary Figure 3B continued



Supplementary Figure 3: Cell frequency differences between MM, MGUS/AM, and healthy control PBMC populations

Plotted are cell frequencies in the particular gates as frequencies of their corresponding parent populations determined with CyTOF. Red squares indicate samples from patients that received MM-specific treatment in the past but not during the last six months. Red lines indicate mean frequencies. (A) Shows scatter plots for the significant frequency differences mentioned in the results and discussion sections. (B) Illustrates population frequency differences for all 244 manual gates analyzed. Control: healthy individuals; * $p<0.05$, ** $p<0.01$. ANOVA and TukeyHSD were used to calculate statistics.

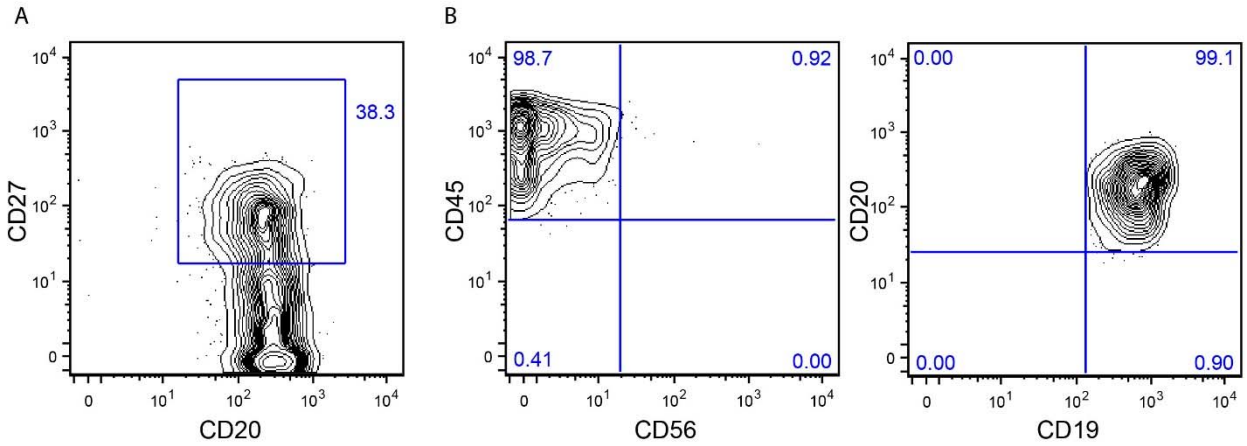
Supplementary Figure 4



Supplementary figure 4: Phenotypic characterization of CD24^{lo}CD38^{+}CD27^{+} B cells in MM samples

All CyTOF plots are pre-gated on CD19^{+}CD20^{+}CD24^{lo}CD38^{+}CD27^{+} B cells. Shown is one example out of n=13 patients.

Supplementary Figure 5

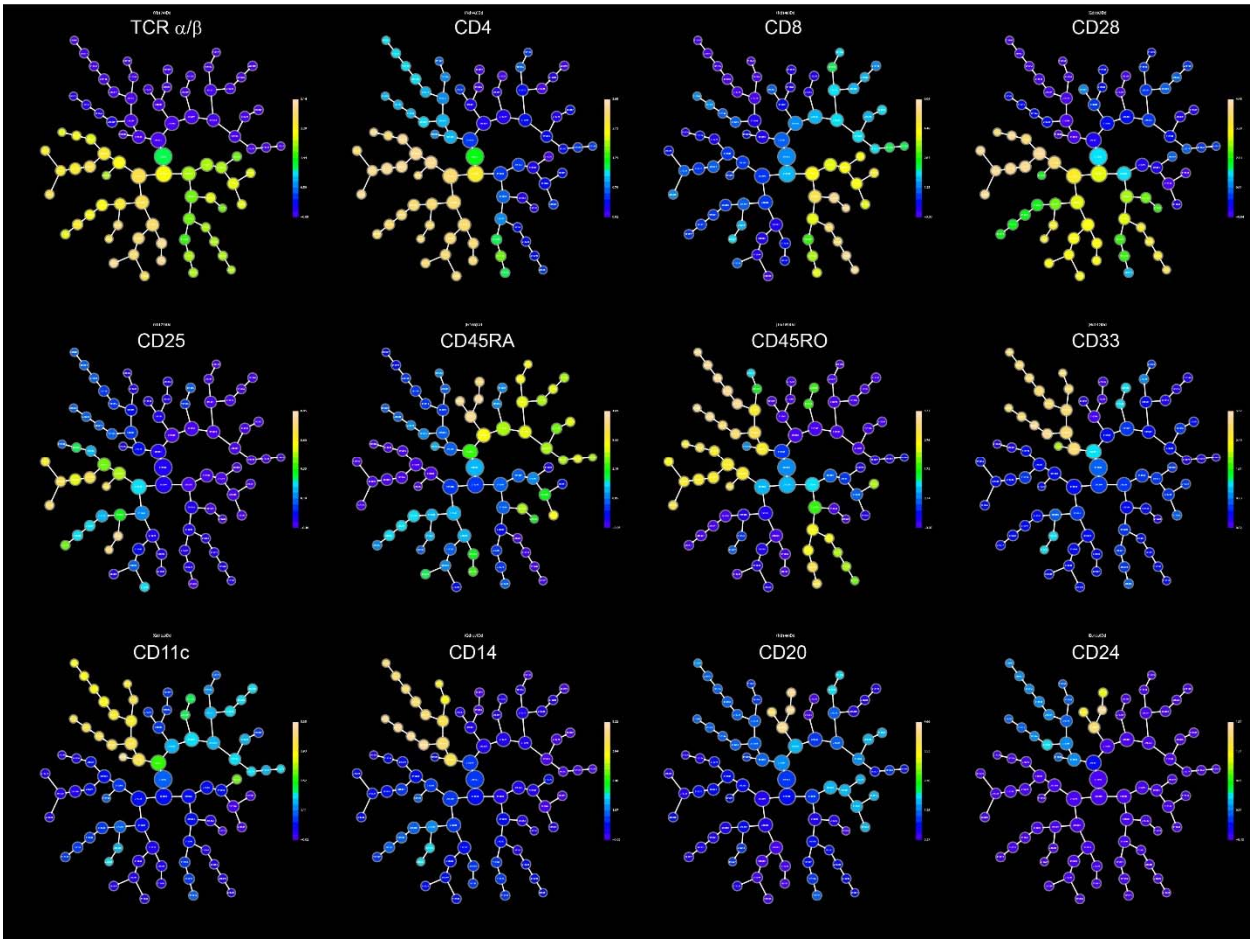


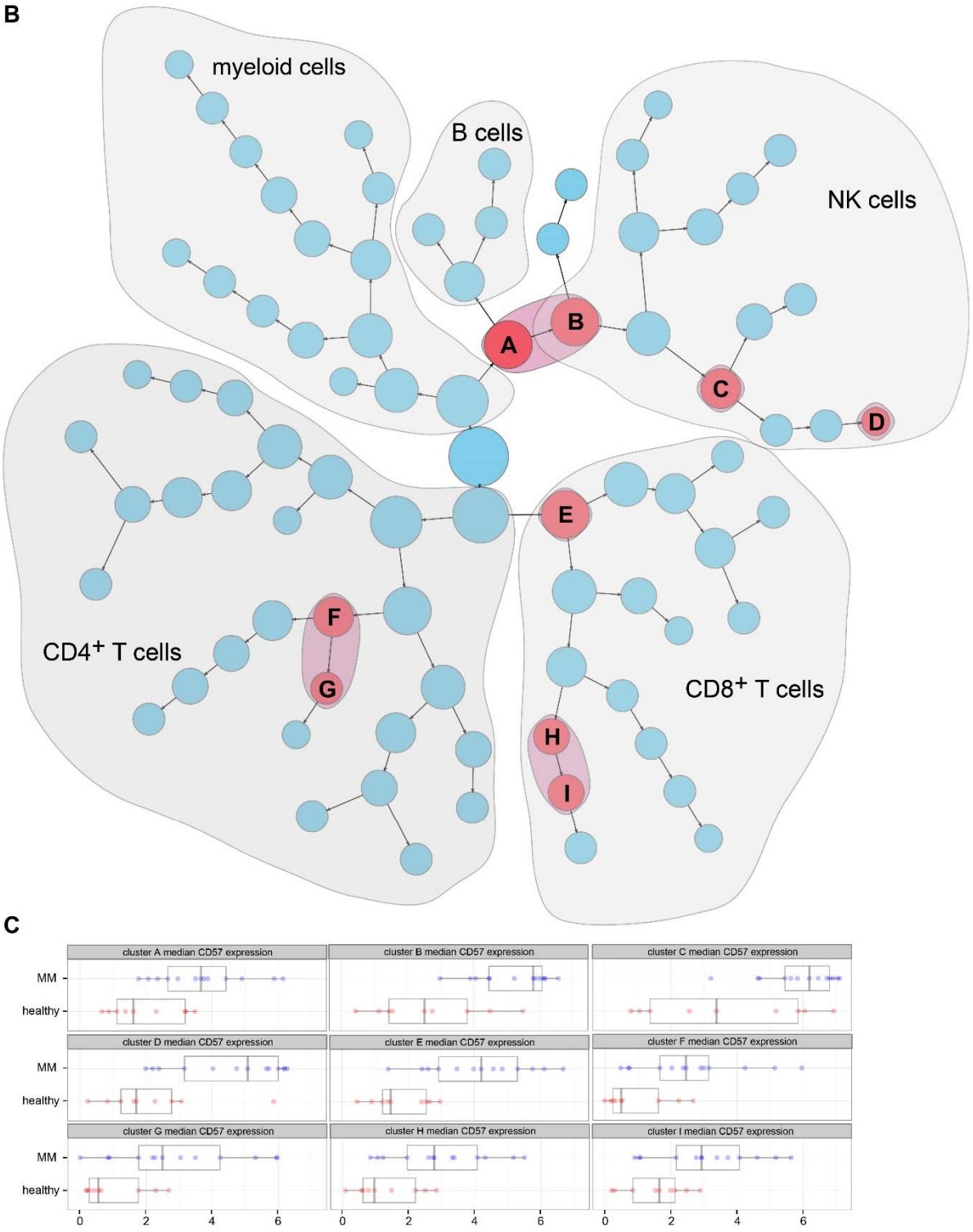
Supplementary Figure 5: CD24^{lo}CD38^{+}CD27^{+} B cells do not show characteristics of malignant plasma cell differentiation

CD24^{lo}CD38^{+}CD27^{+} B cells express CD45, do not express CD56 and are CD20^{+} as determined by CyTOF. (A) is pre-gated on CD19^{+}CD24^{lo}CD38^{+} B cells. (B) shows cells included in the CD27^{+} gate in (A). Shown is one example out of n=13 patients.

Supplementary Figure 6

A



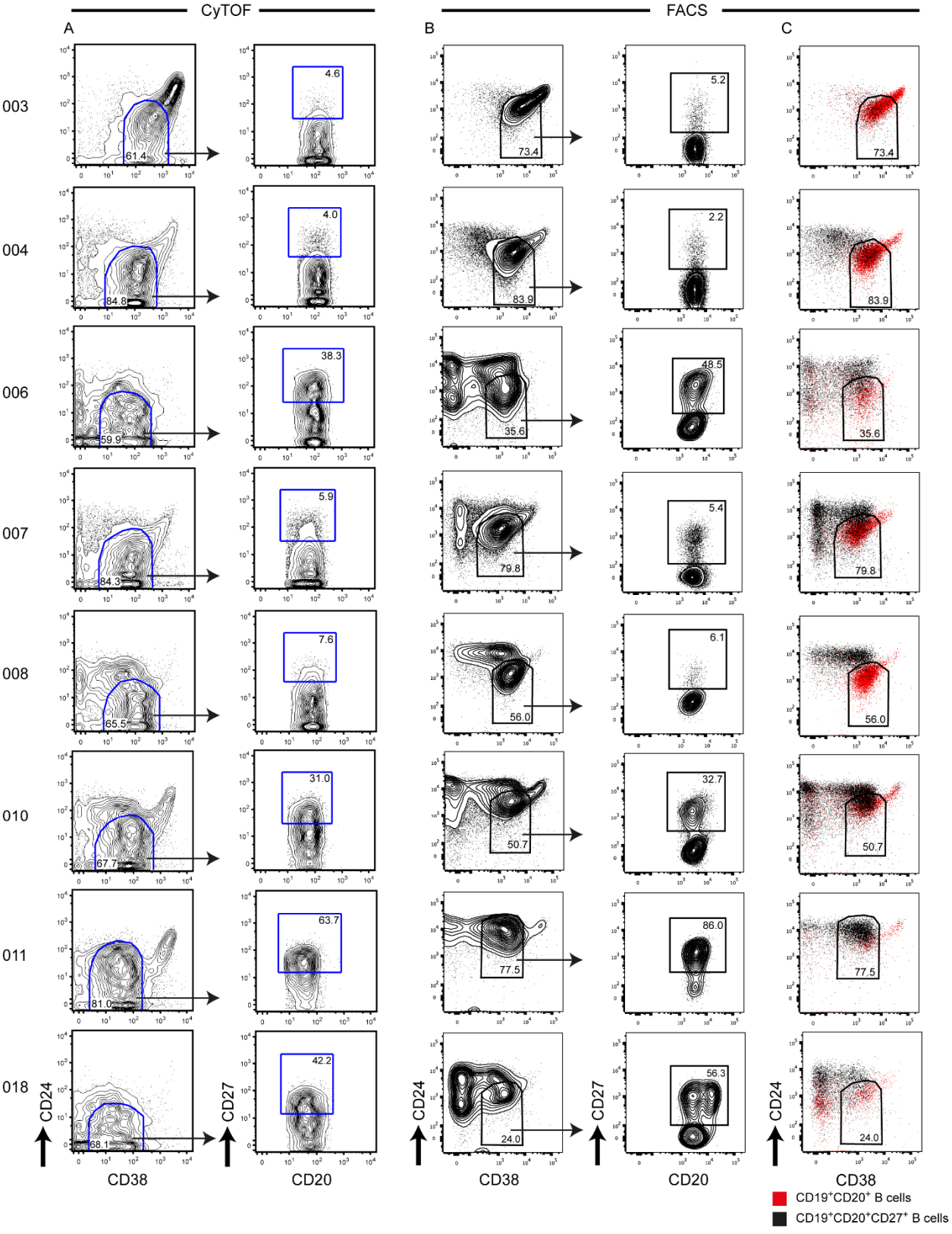


Supplementary Figure 6: Citrus analysis for the comparison of PBMC clusters of MM patients and healthy controls

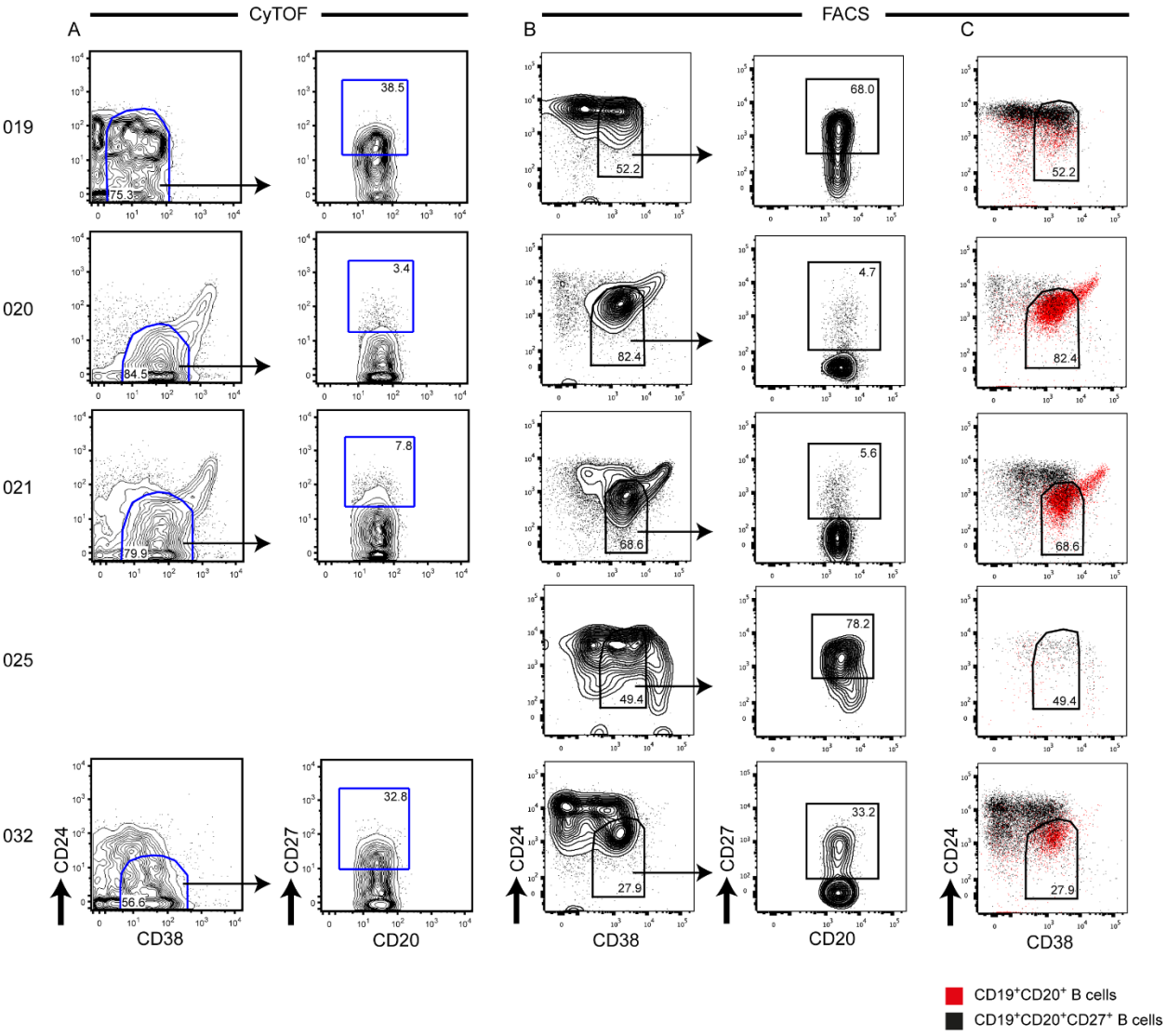
Citrus was run on CyTOF data from all 13 multiple myeloma and 9 healthy control samples. (A) Marker plots for clustering markers. The expression levels of the markers indicated at the top of

each plot are color coded for each individual cluster leading to the identification of major known PBMC populations. (B) Clusters with significantly different (FDR<0.01) CD57 expression between MM patients and healthy individuals are highlighted in red and assigned individual letters. (C) Normalized expression of CD57 on cells in the indicated clusters.

Supplementary Figure 7



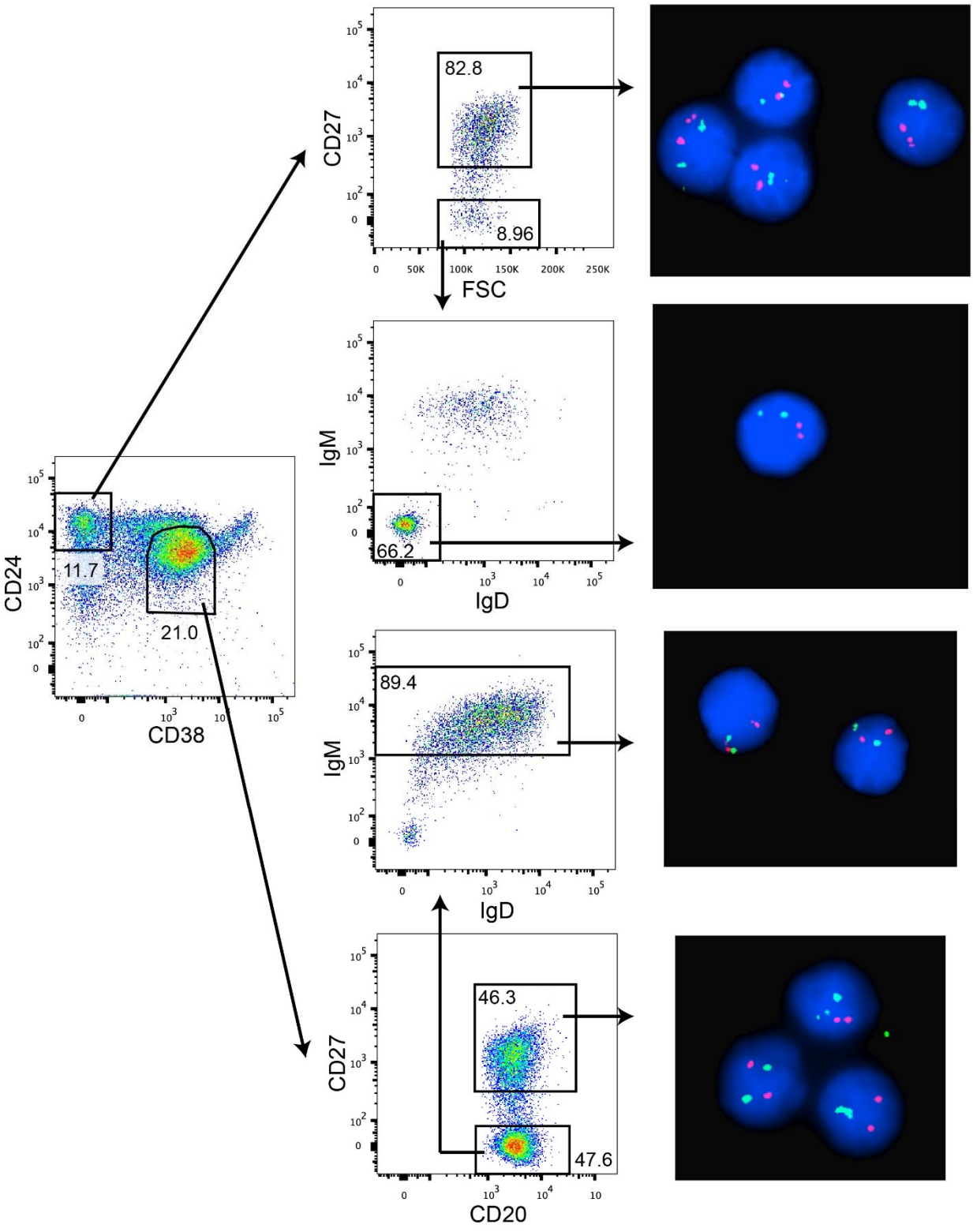
Supplementary Figure 7 continued



Supplementary Figure 7: Mass cytometry and FACS side by side comparison of CD24^{lo}CD38^{+}CD27^{+} B cell populations in MM samples

(A) Shows mass cytometry and (B) FACS staining of cryopreserved PBMCs. For subject 25 we did not have any additional vials of frozen PBMCs available. In contrast to surface FACS staining, mass cytometry staining involves paraformaldehyde fixation and saponin-based permeabilization. All plots are pre-gated on CD19^{+}CD20^{+} B cells and CD27 was gated based on CD27 expression on total CD19^{+}CD20^{+} B cells. In some samples, especially when analyzed with FACS, CD38^{+} B cells can be categorized into CD24^{hi} and CD24^{lo} populations. Whenever distinguishable, the CD24^{lo} population was gated. To illustrate the distribution of CD27^{+} cells among CD38^{+}CD24^{hi} and CD38^{+}CD24^{lo} populations, in (C) we overlaid total CD19^{+}CD20^{+} B cells (red) with total CD19^{+}CD20^{+}CD27^{+} B cells (black). Remaining differences can be attributed to technological differences and low total CD19^{+} B cell numbers (< 1%) in some of the samples. Numbers in the beginning of each line correspond to individual subjects as listed in Table 1. Numbers within the gates indicate percentages of total cells gated of the respective parent populations.

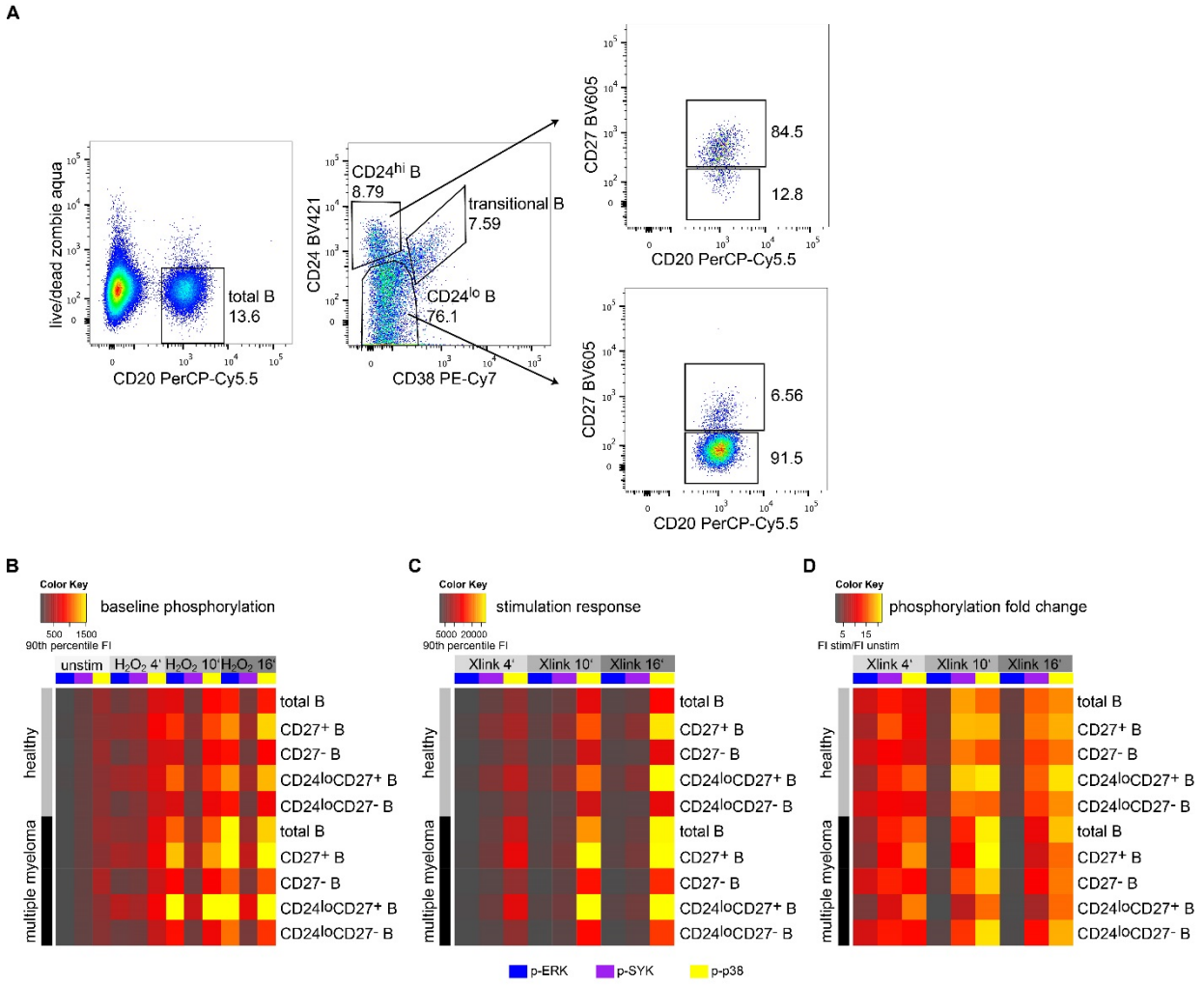
Supplementary Figure 8



Supplementary Figure 8: FISH analysis for t(11;14) in B cell subsets of subject 10 with a t(11;14) positive MM clone

FACS plots are pre-gated on live $CD19^{+}CD20^{+}$ B cells. FISH: orange: CCND1/MYEOV, green: IGH

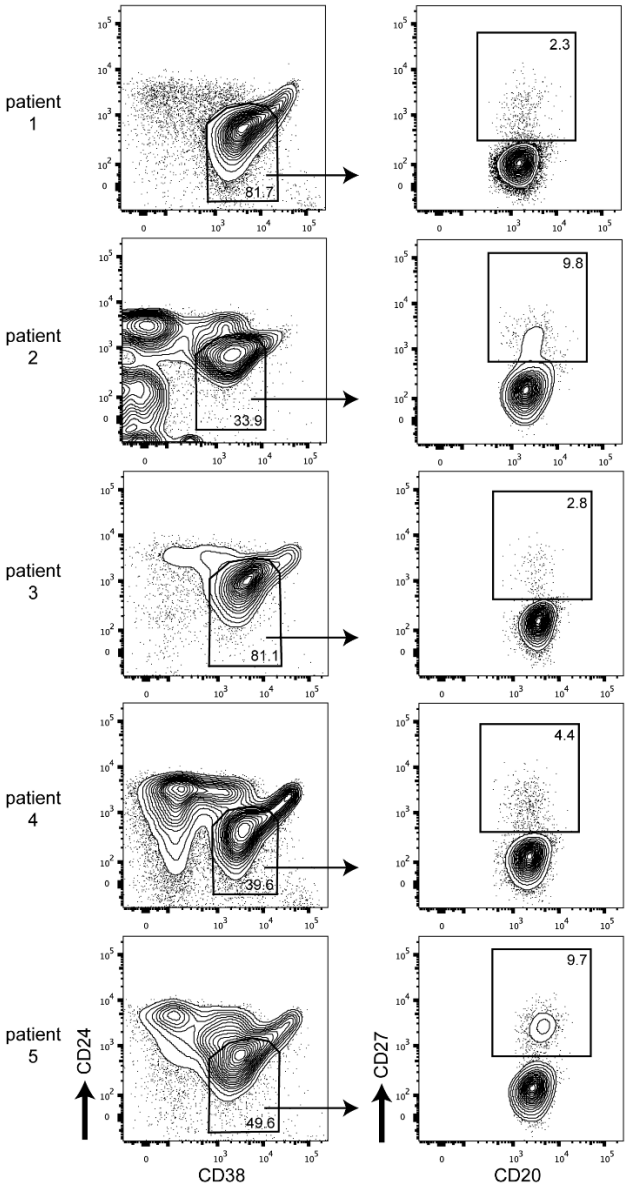
Supplementary Figure 9



Supplementary Figure 9: Validation of phosphorylation responses in two additional MM and healthy control samples

(A) Frequencies of $CD27^+$ B cells in $CD24^{hi}CD38^-$ and $CD24^{lo}CD38^{lo}$ B cell populations in one exemplary healthy individual. $CD24^{hi}CD38^-$ B cells represent $CD24^{hi}CD38^-CD27^+$ memory B cells and $CD24^{lo}CD38^{lo}$ B cells represent $CD24^{lo}CD38^+CD27^-$ naïve B cells as indicated by CD27 expression. (B-D)) Heatmap visualizations of mean phosphorylation levels of n=2 MM patients and n=2 age- and sex-matched healthy individuals (B) at baseline, (C) after BCR stimulation, (D) as calculated respective fold changes (stimulation/baseline levels).

Supplementary Figure 10



Supplementary Figure 10: $CD24^{lo}CD38^{+}CD27^{+}$ B cells in the peripheral blood of colorectal cancer patients

Cryopreserved PBMC of five colorectal cancer patients were analyzed with FACS. All plots are pre-gated on live $CD19^{+}CD20^{+}$ lymphocytes. Patient 1: 70 year old, female, rectal adenocarcinoma, pT3pN1, treatment naïve; Patient 2: 78 year old, female, transverse colon adenocarcinoma, T3pN0, treatment naïve; Patient 3: 78 year old, male, rectal adenocarcinoma, pT3N0, treatment naïve; Patient 4: 51 year old, female, rectal adenocarcinoma, T2N0, treatment naïve; Patient 5: 87 year old, female, sigmoid adenocarcinoma, pT3pN0, treatment naïve