

Phenotypic evolution of circulating plasma cells from early precursor stages to multiple myeloma

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Supplementary Information to

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Supplementary information on methodology

Study population: exclusion criteria

HIV- or hepatitis C infections were exclusion criteria in patients and healthy donors. Patients with monoclonal gammopathy of clinical significance, amyloidosis or patients with additional hematologic malignancies were also excluded.

Patient samples

Peripheral blood mononuclear cells (PBMCs) were enriched by density gradient centrifugation (Sepmate, StemCell Technologies according to manufacturer's protocol and Pancoll, Pan-Biotech) and viably frozen in RPMI1640 medium (ThermoFisher) supplemented with 20% fetal bovine serum (FBS) and 10% DMSO (AppliChem).

Analysis of Multiparameter spectral flow-cytometry.

All datasets were processed using the Cytolution software platform (from Cytolytics GmbH), which implements a flexible pipeline consisting of data preprocessing followed by downstream analytical workflows. The pipeline is designed to standardize data quality, reduce technical variability, and ensure robust performance of downstream algorithms. Signal intensities were transformed on a per-channel basis using an inverse hyperbolic sine (asinh) transformation: $ft(x)=asinh((x-Poffset)/Pwidth)$.

Transformation parameters (Poffset and Pwidth) were estimated running an algorithmic optimization procedure. Scatter parameters were excluded from asinh transformation and instead scaled linearly, while the time channel was excluded entirely. All transformation parameters were retained and made available for inspection or manual adjustment.

To mitigate systematic technical variation across samples, a batch correction step was applied. The effectiveness of batch correction was evaluated using pre- and post-correction

visualizations, allowing assessment of residual batch effects and potential overcorrection. For analysis of marker expression according to their respective MFI, semi-automated cluster exploring was applied.

Flow Cytometry Antibodies

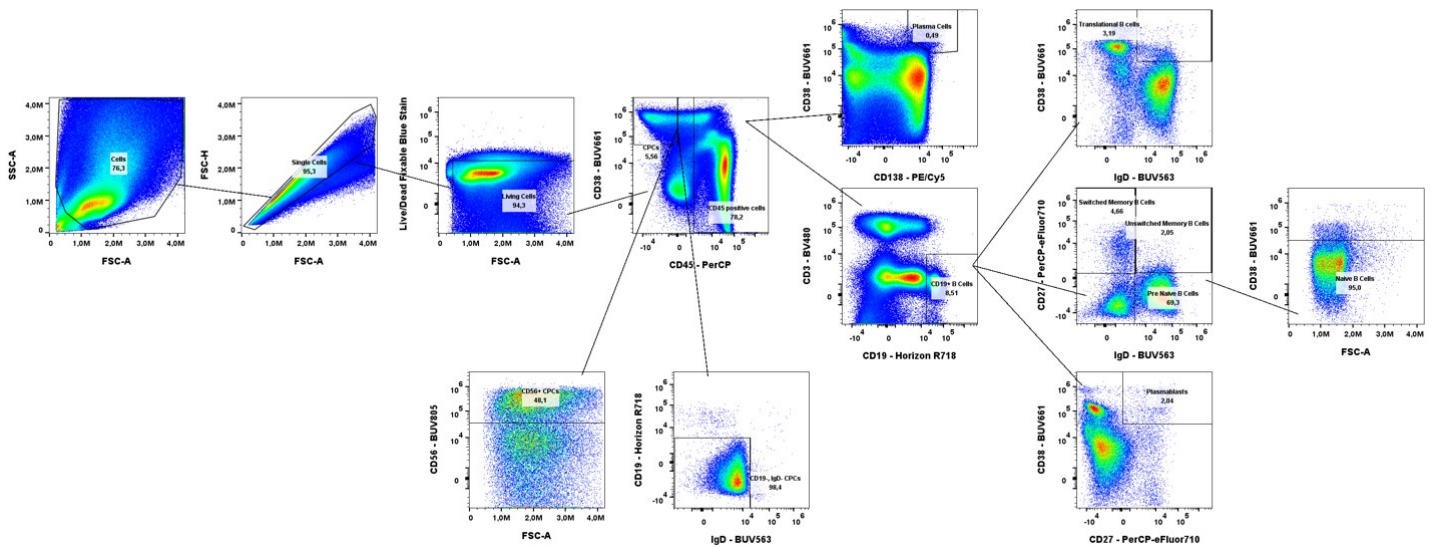
All anti-human antibodies were titrated to determine the optimal concentration prior to the staining of patient samples. For this purpose, cell line LP-1 (ACC 41, RRID:CVCL_0012) was obtained from DSMZ and tested negative for mycoplasma contamination, moreover PBMCs were used. Antibodies and viability dye were purchased from either BioLegend (BL), BD Biosciences (BD), ThermoFisher Scientific (Thermo), or Novus Biologicals (Novus) under the following catalog numbers: CD45 (BL, #304026, RRID:AB_893337), CD3 (BD, #750981, RRID:AB_2875050), TCR $\gamma\delta$ (BD, #655410, RRID:AB_2870377), CD45RA (BD, #740806, RRID:AB_2740469), CCR7 (BL, #353264, RRID:AB_2894483), CD56 (BD, #749086, RRID:AB_2873478), CD16 (BL, #302068, RRID:AB_2876587), CD14 (Thermo, #H007T02B05, RRID:AB_3098075), HLA-DR (BD, #564231, RRID:AB_2738685), HLA-ABC (BL, #311406, RRID:AB_314875), CD19 (BD, #567344, RRID:AB_2916561), CD11c (BL, #301628, RRID:AB_11203895), CD123 (BL, #306012, RRID:AB_439779), CD20 (BL, #302332, RRID:AB_2563805), IgD (BD, #741394, RRID:AB_2870889), CD27 (Thermo, #46-0279-41, RRID:AB_1834392), CD38 (BD, #612970, RRID:AB_2916888), CD138 (BL, #356548, RRID:AB_2894487), BCMA (BL, #357528, RRID:AB_2890787), CD8 (BD, #741199, RRID:AB_2870759), CD2 (BD, #745205, RRID:AB_3684781), CD4 (BL, #344672, RRID:AB_2894479), CCR6 (BD, #612780, RRID:AB_2870109), CD307e (BD, #749606, RRID:AB_2873908), CCR4 (BL, #359436, RRID:AB_2894491), CXCR3 (BL, #353714, RRID:AB_10962908), CD25 (BL, #302618, RRID:AB_493045), TIGIT (BL, #372734, RRID:AB_2876700), PD-1 (Thermo, #62-2799-42, RRID:AB_2784827), TIM-3 (BD, #752363, RRID:AB_2875880), CD40L (BL, #310842, RRID:AB_2572187), CD127 (BD, #742547, RRID:AB_2740857), CD69 (Novus, #NBP1-43387AF532, RRID:AB_3209264), LIVE/DEAD™ Fixable Blue Dead Cell Staining dye (Thermo, #L34961).

Statistics

Effect sizes included eta squared/ η^2 (one-way ANOVA), epsilon squared/ ϵ^2 (Welch/Brown–Forsythe ANOVA and K-W), d_z (paired t-tests), Hedges' g_w (Welch's t-test) and r_{fb} (Mann–Whitney U), with 95% confidence intervals where applicable.

Supplementary Figures

Supplementary Figure 1. Gating Strategy for the identification of CD56⁺ circulating tumour plasma cells (CTPCs).

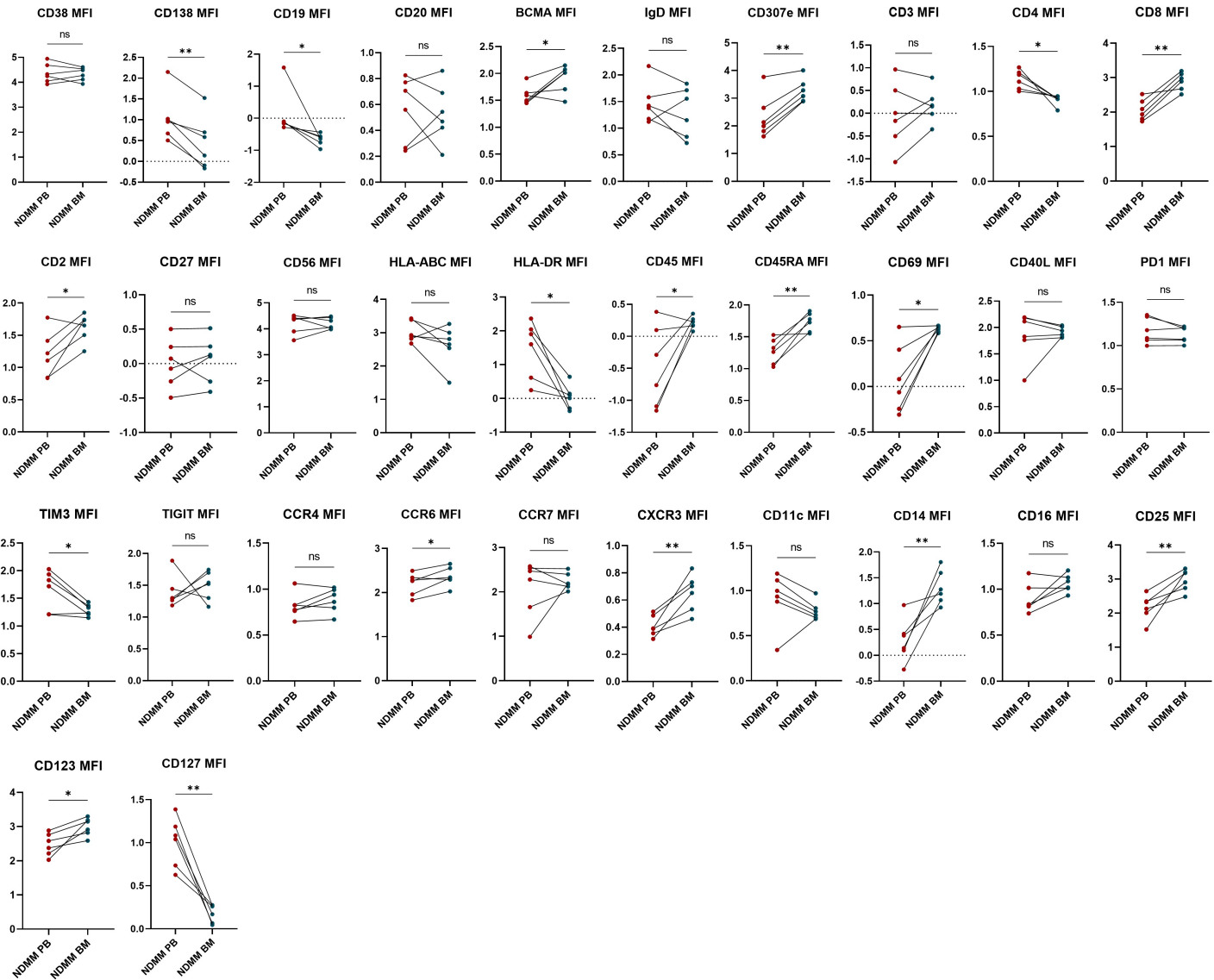


Supplementary Figure 2. Additional boxplots of each marker across disease stages.

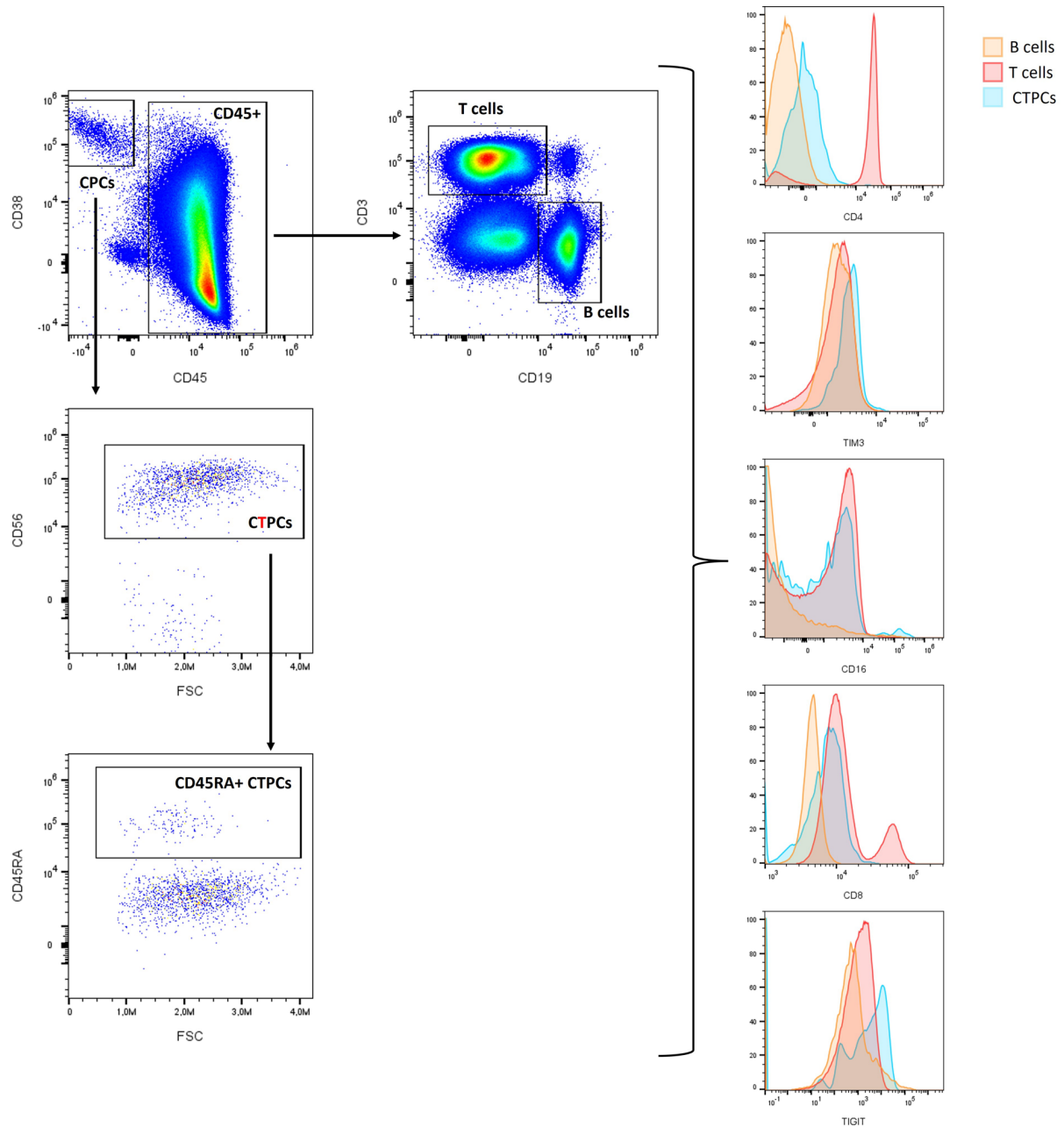
Transformed mean fluorescence intensity (MFI) values on CD56⁺ CTPCs at different disease stages. Data is presented as box-and-whisker plot with median values indicated. Statistical significance was determined via ANOVA or Kruskal Wallis with post hoc FDR correction. Asterisks mark a statistically significant discovery, ns = no statistically significant discovery.



Supplementary Figure 3. Paired dot-plots of each marker comparing peripheral blood and bone marrow samples. Transformed mean fluorescence intensity (MFI) values (Y-axis) of markers on abnormal, CD56⁺ PC in paired BM and PB samples. Paired samples are connected by a line. Statistical significance was determined via paired t-test (for normally distributed data) or Wilcoxon test (for nonparametric data). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



Supplementary Figure 4. Exemplary gating of a newly diagnosed multiple myeloma (NDMM) sample. The figure shows the gating strategy for circulating plasma cells (CPCs), followed by analysis of CD56 and CD45RA expression. Lineage marker expression is presented as histograms with overlays depicting the expression on B cells, T cells, and CD56⁺ circulating tumour plasma cells (CTPCs).



Supplementary Tables

Supplementary Table 1. Overview of the applied antibodies for the study. For each antibody, clone, providing company, and fluorophore are shown. All antibodies have been titrated on respective antigen-expressing control cells prior to the staining of patient cells.

Antigen	Clone	Fluorophor	Company	Catalog No.
CD45	HI30	PerCP*	BioLegend	304026
CD3	OKT3	BV480	BD Biosciences	750981
TCR $\gamma\delta$	B1	PE-Cy7	BD Biosciences	655410
CD45RA	5H9	BV711	BD Biosciences	740806
CCR7	G043H7	APC-Fire 810	BioLegend	353264
CD56	NCAM16.2	BUV805	BD Biosciences	749086
CD16	3G8	PE-Fire 640	BioLegend	302068
CD14	MEM-15	NovaFlour Blue610	ThermoFisher	H007T02B05
HLA-DR	G46-6	BV650	BD Biosciences	564231
HLA-ABC	w6/32	PE	BioLegend	311406
CD19	HIB19	Horizon R718	BD Biosciences	567344
CD11c	3.9	BV421	BioLegend	301628
CD123	6H6	APC	BioLegend	306012
CD20	2H7	BV570	BioLegend	302332
IgD	IA62	BUV563	BD Biosciences	741394
CD27	O323	PerCP-e Fluor 710	ThermoFisher	46-0279-41
CD38	HIT2	BUV661	BD Biosciences	612970
CD138	MI15	PE-Cy5	BioLegend	356548
BCMA	19F2	KIRAVIA Blue 520	BioLegend	357528
CD8	SK1	BUV496	BD Biosciences	741199
CD2	S5.2	BV605	BD Biosciences	745205
CD4	SK3	Spark YG 593	BioLegend	344672
CCR6	11A9	BUV737	BD Biosciences	612780
CD307e	509F6	BV510	BD Biosciences	749606
CCR4	L291H4	PE-Fire 700	BioLegend	359436
CXCR3	G025H7	PerCP-Cy5.5	BioLegend	353714
CD25	BC96	AF647	BioLegend	302618
TIGIT	A15153G	APC-Cy7	BioLegend	372734
PD-1	J105	Super Bright 436	ThermoFisher	62-2799-42

TIM-3	7D3	BUV615	BD Biosciences	752363
CD40L	24-31	BV785	BioLegend	310842
CD127	HIL-7R-M21	BUV395	BD Biosciences	742547
CD69	FN50	Alexa Fluor 532	Novus Biologicals	NBP1-43387AF532
LIVE/DEAD™ Fixable Blue Dead Cell Staining dye	-	-	ThermoFisher	L34961

Supplementary Table 2. Differential expression of CTPC surface markers across disease stages. Overall *P*-values are shown for group comparisons, with pairwise comparisons between healthy controls, MGUS, SMM, and NDMM provided with adjusted *P*-values. Adjusted *P*-values < 0.05 are marked in bold.

Marker	Overall <i>P</i>	Comparison	q / adjusted <i>P</i>
CD38	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	<0.001 0.004 0.083 0.004 <0.001 0.048
CD138	0.712	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.965 >0.999 >0.999 0.965 0.965 >0.999
CD19	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	<0.001 <0.001 <0.001 0.099 <0.001 <0.001
CD20	0.087	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.993 0.340 0.633 0.169 0.510 0.100
BCMA	0.950	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM	0.988 0.988 0.988 0.988 0.988

		SMM vs. NDMM	0.988
IgD	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	<0.001 0.003 0.236 0.102 <0.001 0.003
CD307e	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.101 0.464 0.123 0.005 <0.001 0.133
CD3	0.006	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.279 0.830 0.174 0.215 0.002 0.098
CD4	0.002	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.003 0.011 0.196 0.327 0.014 0.051
CD8	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.083 0.010 <0.001 0.031 <0.001 <0.001
CD2	0.144	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.943 0.943 0.268 0.943 0.138 0.268
CD27	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	<0.001 <0.001 <0.001 0.341 0.196 0.313
HLA-ABC	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.002 0.099 0.552 0.034 <0.001 0.038
HLA-DR	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM	<0.001 <0.001 0.009 0.185

		MGUS vs. NDMM SMM vs. NDMM	0.127 0.047
CD45	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	<0.001 0.002 <0.001 0.007 0.134 0.099
CD45RA	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.034 <0.001 <0.001 0.002 <0.001 <0.001
CD69	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	<0.001 0.003 0.144 0.017 <0.001 0.014
CD40L	0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.071 0.638 0.005 0.040 0.040 0.002
PD1	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	<0.001 0.002 0.126 0.036 <0.001 0.011
TIM3	0.023	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.093 0.103 0.012 0.589 0.103 0.103
TIGIT	0.004	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.119 0.257 0.257 0.289 0.002 0.027
CCR4	0.031	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.024 0.011 0.011 0.248 0.248 0.437
CCR6	0.007	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM	0.585 0.585 0.050

		MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.665 0.004 0.005
CCR7	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.341 0.341 <0.001 0.488 <0.001 <0.001
CXCR3	0.534	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.718 0.718 0.718 0.718 0.718 0.718
CD11c	0.008	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.003 0.037 0.016 0.209 0.524 0.393
CD14	0.010	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.056 0.173 0.777 0.386 0.021 0.103
CD16	0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.003 0.007 0.206 0.382 0.011 0.033
CD25	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.003 0.011 0.225 0.225 <0.001 <0.001
CD123	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	<0.001 0.007 0.220 0.038 <0.001 0.010
CD127	<0.001	Healthy vs. MGUS Healthy vs. SMM Healthy vs. NDMM MGUS vs. SMM MGUS vs. NDMM SMM vs. NDMM	0.008 0.071 <0.001 0.068 0.006 <0.001
CD56	<0.001	Healthy vs. MGUS Healthy vs. SMM	<0.001 <0.001

		Healthy vs. NDMM	<0.001
		MGUS vs. SMM	0.037
		MGUS vs. NDMM	0.003
		SMM vs. NDMM	0.081