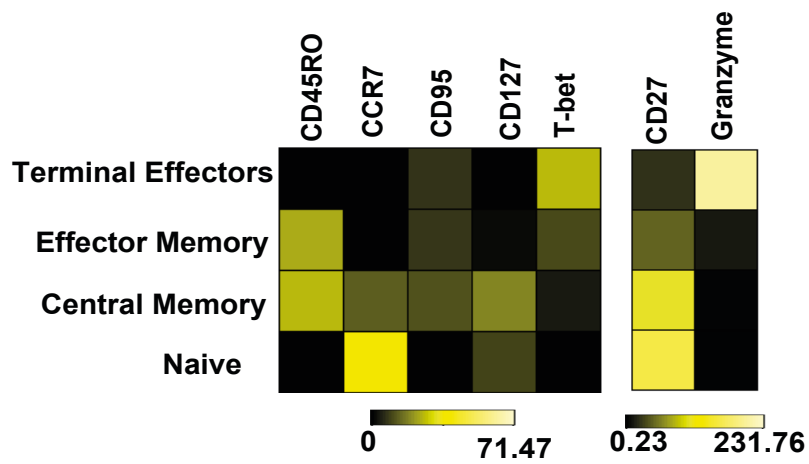


Supplementary data

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FigS1: Heatmap of CD45RO, CCR7, CD95, CD127, T-bet, CD27 and Granzyme B expression in terminal effectors, effector memory, central memory and naïve CD8 T cells (Representative data from MM patient).

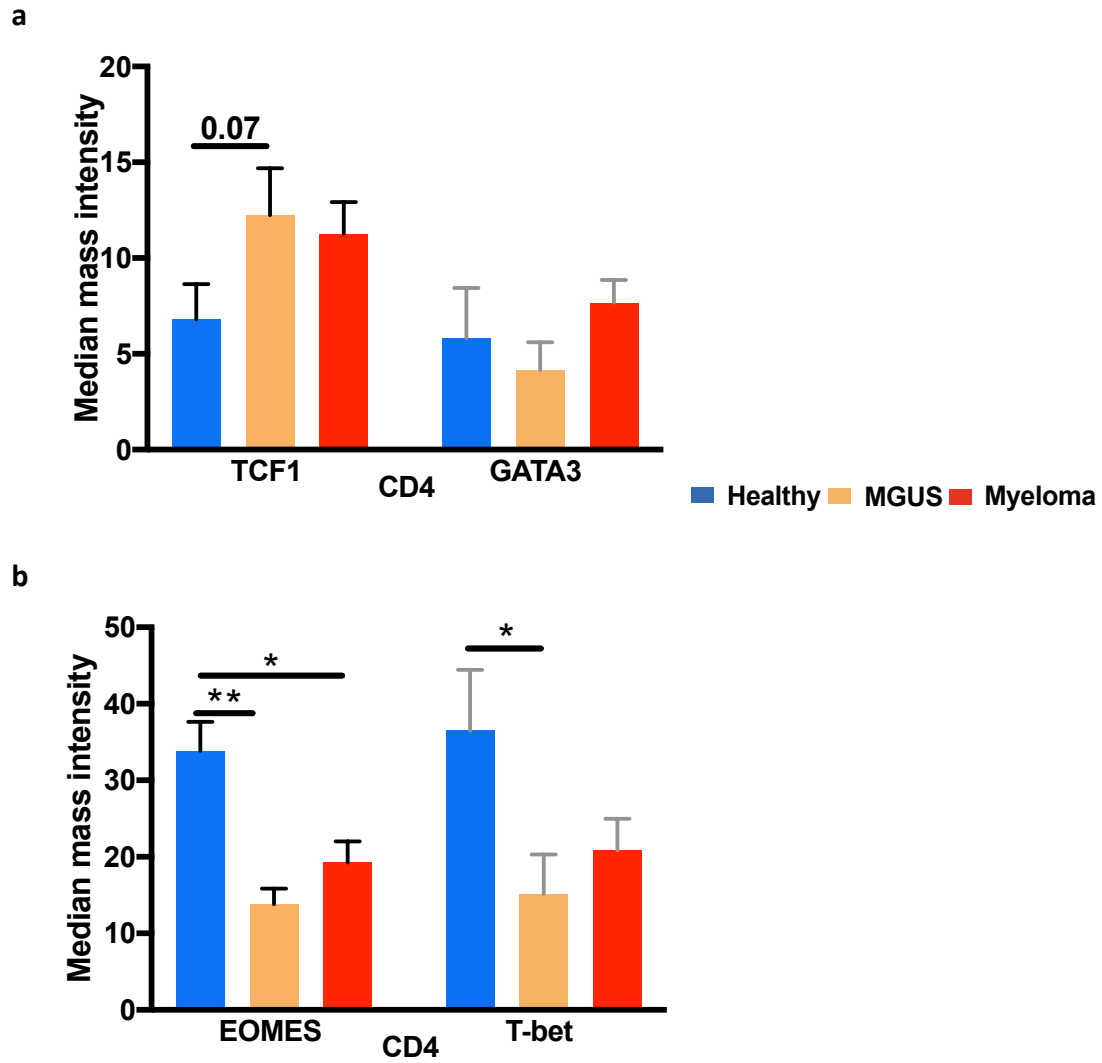


Fig S2: Expression of transcription factors in CD4 memory T cells in healthy donors and patients with MGUS & myeloma. **a.** Median mass intensity of TCF1 and GATA3. **b.** Median mass intensity of EOMES and T-bet.

* $p < 0.05$, ** $p < 0.01$

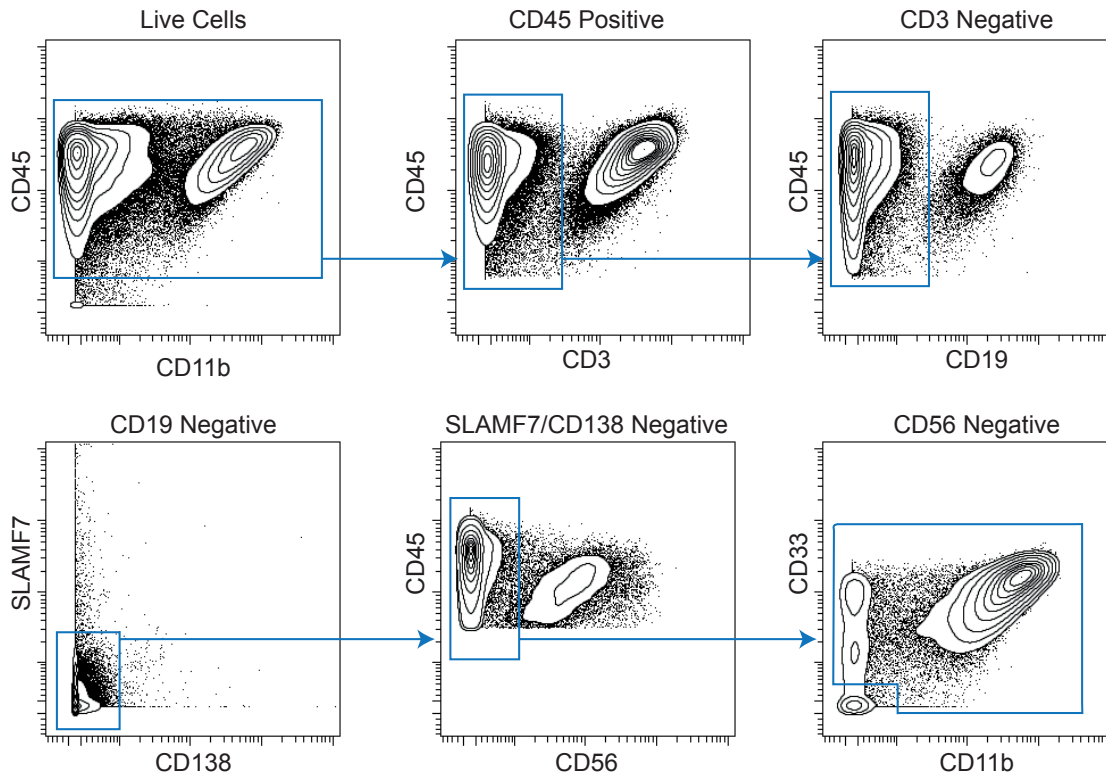


Fig S3: Gating strategy for myeloid cell analysis by CyTOF. Bulk myeloid cells were identified after excluding CD3, CD19, CD138, SLAMF7, CD56. Bulk myeloid cells were identified as CD33 or CD11b positive.

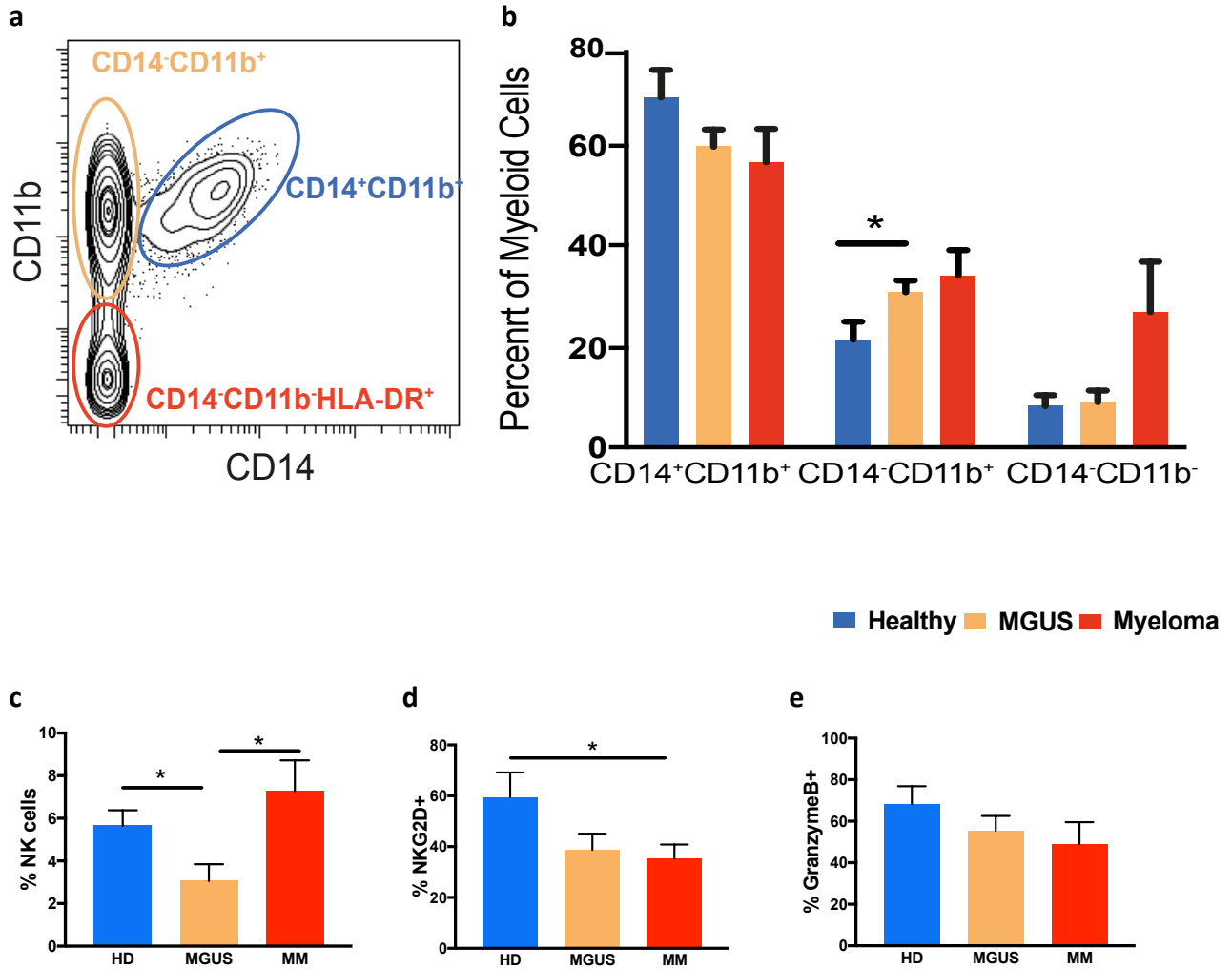


Fig S4: Characteristics of myeloid cells and NK cells in healthy, MGUS and MM. **a.** Biaxial gating of mass cytometry data for CD14⁺CD11b⁺, CD14-CD11b⁺, and CD14-CD11b⁻ subsets from myeloid cells. **b.** Percent abundance of subsets from total myeloid cells. **c.** Percent of NK cells (from CD45^{hi}) in healthy, MGUS and MM. **d, e.** Percent of CD56dim expressing NKG2D and Granzyme.

* p<0.05

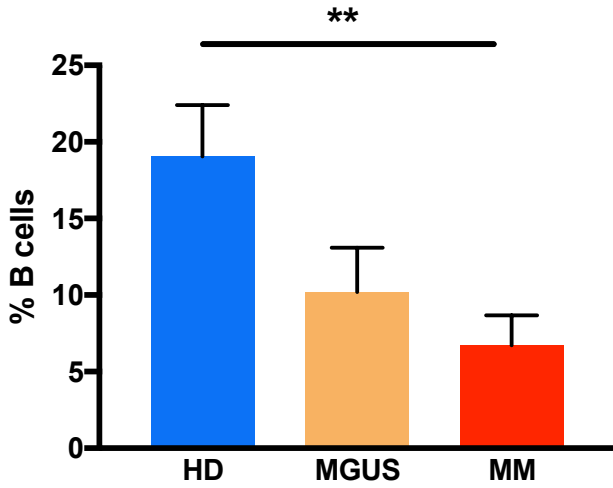
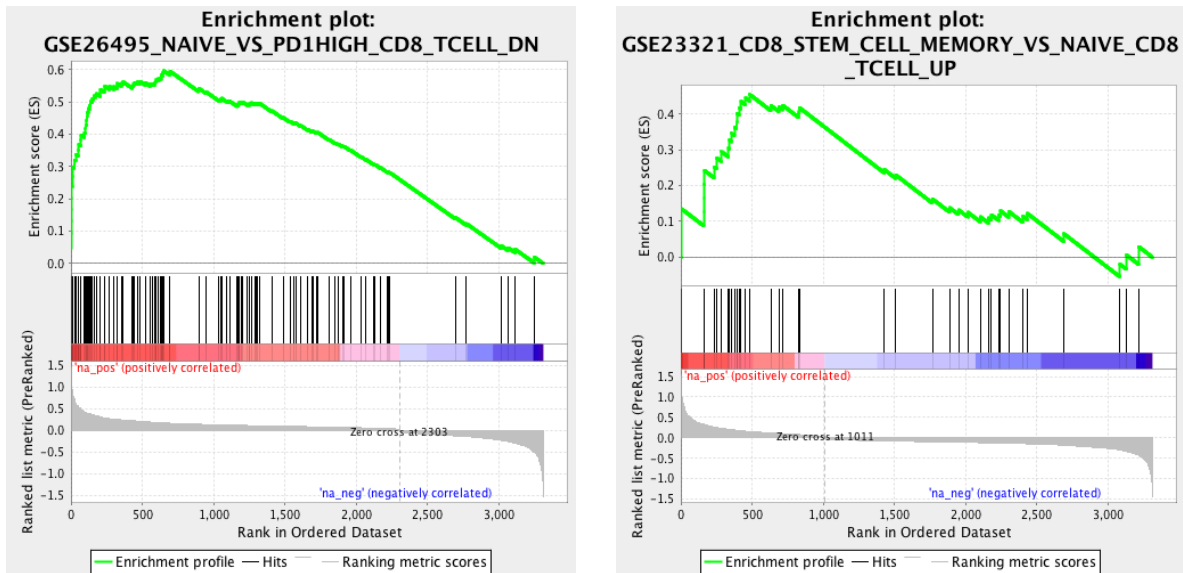


Fig S5: Figure shows B cells as percent of CD45+ lymphocytes in healthy donor, MGUS and MM patients as analyzed by mass cytometry.

** p<0.01



Gene Set ID	Enriched Cluster	Description	Nominal p-value	PMID
GSE26495 (shown above left)	T3	Genes down-regulated in naïve vs PD-1 high CD8 T cells	p<0.001	21383243
GSE23321 (shown above right)	T2	Genes up-regulated in CD8 stem cell memory vs naïve CD8 T cells	p<0.001	21926977
GSE45739	T3	Genes down-regulated in unstimulated vs activated CD4 T cells	p<0.001	23755101
GSE3982	T3	Genes up-regulated in effector vs central memory CD4 T cells	p<0.001	16474395
GSE11057	T3	Genes down-regulated in naïve vs effector memory T cells	p<0.001	19568420

Fig S6: GSEA pathway analysis of differential gene expression between cluster T3 (enriched in myeloma) and cluster T2 (enriched in MGUS) revealed enrichment of gene sets associated with stimulation, activation, and effector phenotype in cluster T3 relative to T2, and enrichment of a gene set associated with stem cell memory T cells in cluster T2 relative to T3. Gene signatures were obtained from the Molecular Signatures Database (Subramanian, Aravind, et al. "Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles." *Proceedings of the National Academy of Sciences* 102.43 (2005): 15545-15550.).

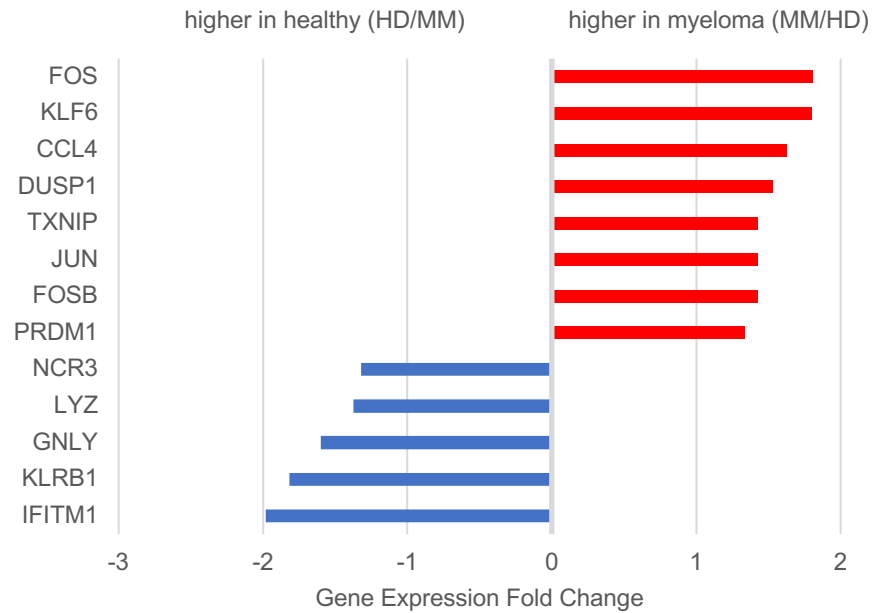
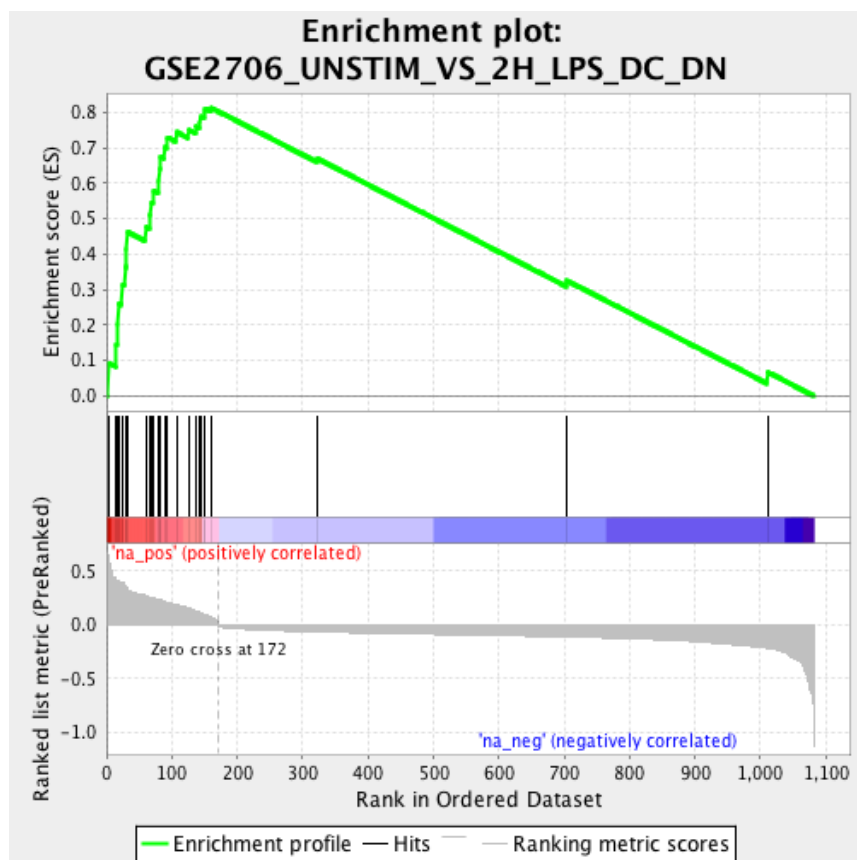


Fig S7: Gene expression fold-change of selected highly differentially expressed genes in T cell cluster T3 between myeloma and healthy samples. Red=increased in myeloma (fold change calculated as myeloma/healthy), blue=increased in healthy (fold change calculated as healthy/myeloma). Myeloma T cells exhibit enrichment in genes associated with exhaustion (PRDM1) and activation (CCL4) and downregulation of lysozyme and Granulysin (indicative of dysfunction).



Gene Set ID	Description	Nominal p-value	PMID
GSE2706 (shown above)	Genes down-regulated in unstimulated vs LPS-stimulated dendritic cells	$p < 0.001$	15995707
GSE22886	Genes down-regulated in unstimulated vs LPS-stimulated dendritic cells	$p < 0.001$	15789058
GSE9988	Genes up-regulated in monocytes treated with anti-TREM1 and LPS vs monocytes treated with control IgG	$p < 0.001$	18292579
GSE2706	Genes down-regulated in comparison of unstimulated vs R848-stimulated dendritic cells.	$p < 0.001$	15995707

Fig S8: GSEA pathway analysis of differential gene expression between MGUS and myeloma myeloid cells revealed enrichment of gene sets associated with myeloid cell stimulation by TLR agonists in MGUS myeloid cells relative to myeloma myeloid cells. Gene signatures were obtained from the Molecular Signatures Database (Subramanian, Aravind, et al. "Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles." *Proceedings of the National Academy of Sciences* 102.43 (2005): 15545-15550.).

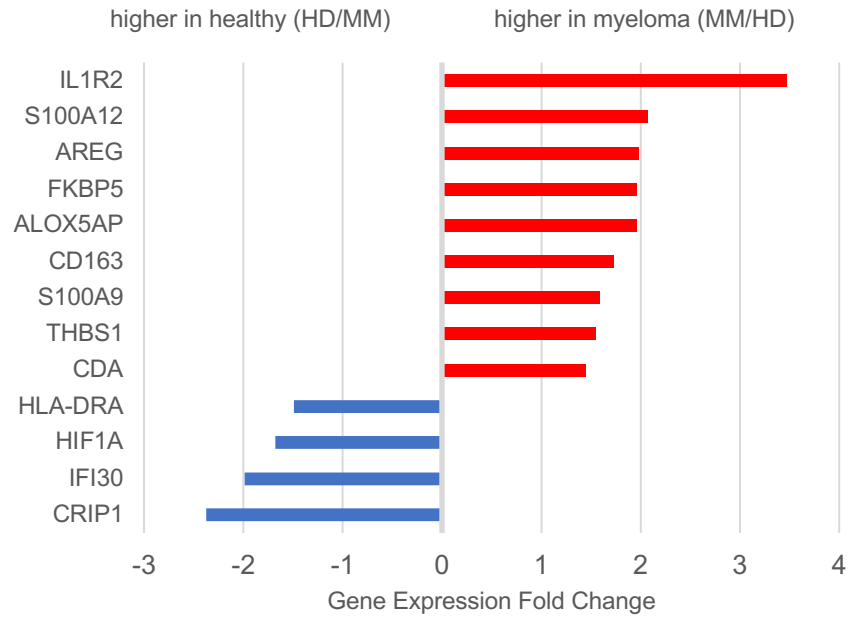
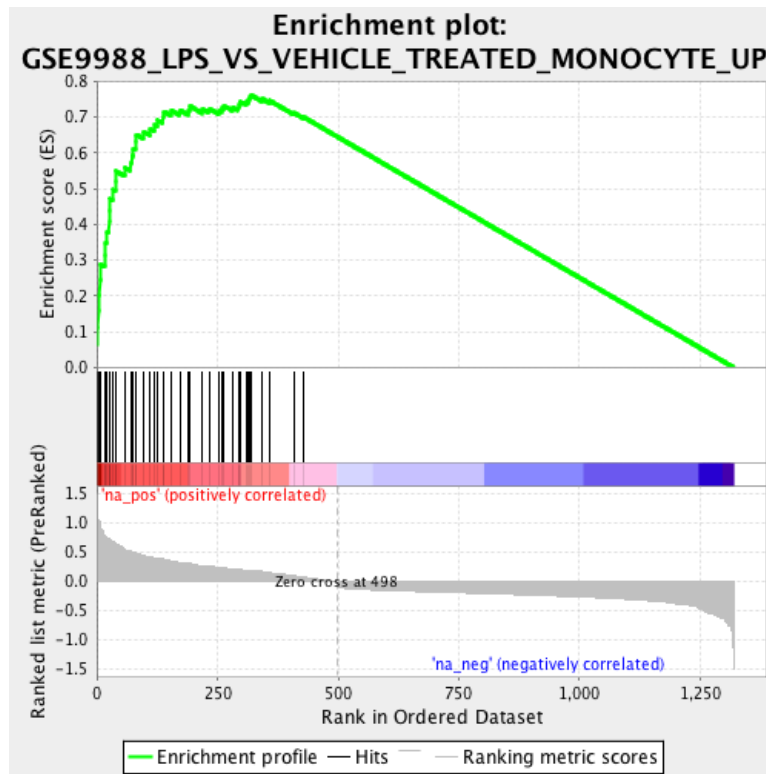


Fig S9: Selected highly differentially expressed genes in myeloid cluster M1 (monocytes) between myeloma and healthy samples. Red=increased in myeloma (fold change calculated as myeloma/healthy), blue=increased in healthy (fold change calculated as healthy/myeloma). Myeloma myeloid cells exhibit a more immune suppressive phenotype.



Gene Set ID	Description	Nominal p-value	PMID
GSE9988 (shown above)	Genes up-regulated in comparison of LPS-treated vs untreated monocytes	$p < 0.001$	18292579
GSE2706	Genes down-regulated in comparison of unstimulated vs LPS-stimulated dendritic cells.	$p < 0.001$	15995707
GSE9988	Genes up-regulated in monocytes treated with anti-TREM1 and LPS vs monocytes treated with control IgG	$p < 0.001$	18292579
GSE2706	Genes down-regulated in comparison of unstimulated vs LPS- and R848-stimulated dendritic cells.	$p < 0.001$	15995707

Fig S10: GSEA pathway analysis of differential gene expression between myeloid cells from patients with higher than mean proportion of cluster T2 (stem/resident-like T cells) and myeloid cells from all other patients revealed enrichment of gene sets associated with myeloid cell stimulation by TLR agonists in T2-enriched patient myeloid cells relative to other patient myeloid cells. Gene signatures were obtained from the Molecular Signatures Database (Subramanian, Aravind, et al. "Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles." *Proceedings of the National Academy of Sciences* 102.43 (2005): 15545-15550.).

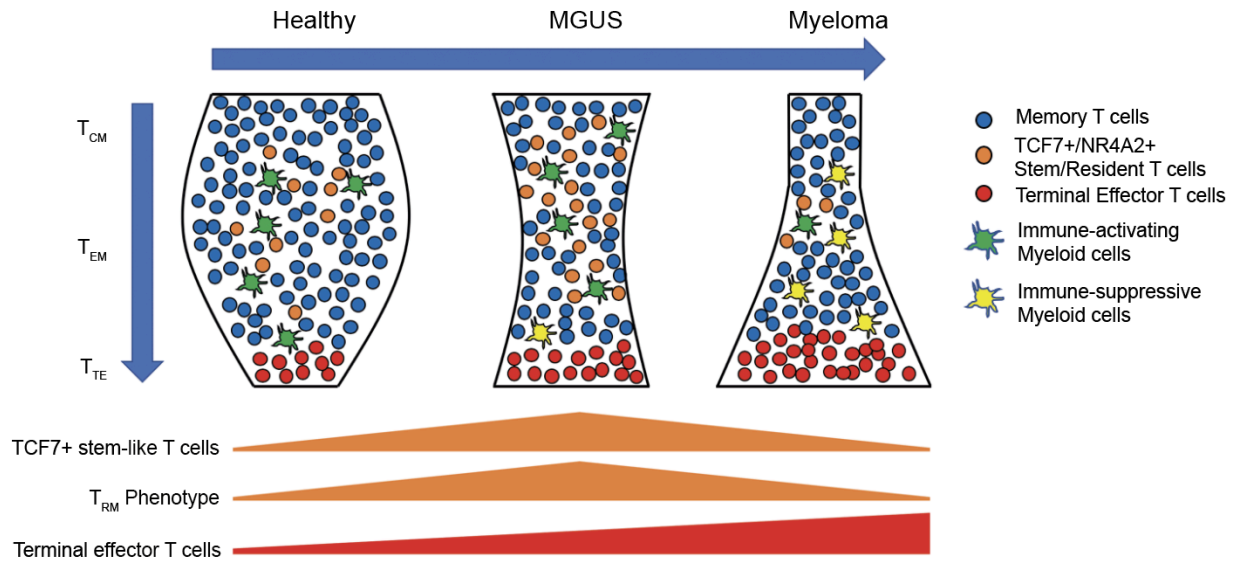


Fig S11: Schematic of immune cell population changes over myeloma disease progression. Left to right: disease progression from healthy to MGUS to myeloma. Top to bottom: T cell changes from central memory to effector memory to terminal effector. Compartment width corresponds to relative abundance of T cell type as seen by mass cytometry. Central memory and effector memory T cells decrease from healthy to MGUS to myeloma. Meanwhile, an increase in terminal effector T cells is noted as disease progresses, and myeloid cells trend toward an immune-suppressive phenotype. In MGUS, an increase in a stem/resident-like T cell population is seen relative to healthy and myeloma.

Table S1: Patient characteristics

	MGUS (n=15)	MM (n=11)	Healthy (n=12)
Median Age	59 years	60 years	53.5 years
Sex (Male: Female)	9:6	8:3	9:3
M protein IgH isotype			NA
IgG	9	7	
IgA	3	3	
IgM	1	-	
Light chain only	2	1	
M protein IgL isotype			NA
Kappa	11	7	
Lambda	4	4	
ISS Stage	NA		NA
1		3	
2		3	
3		5	

NA: Not applicable

Table S2: List of antibodies and clones used for CyTOF analysis

	Antibody	Clone	Supplier
1	41BB	4B4-1	Biologend
2	B7H3	MIH42	Biologend
3	BDCA2	201A	Fluidigm
4	BDCA3	1A4	Fluidigm
5	c-Kit	104.D2	Biologend
6	CCR7	G043H7	Fluidigm
7	CD103	Ber-ACT8	Fluidigm
8	CD11b	ICMF44	Fluidigm
9	CD11c	Bu15	Fluidigm
1	CD123	6H6	Fluidigm
11	CD127	A019D5	Fluidigm
12	CD138	M15	Biologend
13	CD14	RM052	Fluidigm
14	CD15	W6D3	Fluidigm
15	CD155	SKIL4	Biologend
16	CD16	3G8	Fluidigm
17	CD163	GHI/61	Fluidigm
18	CD19	HIB19	Fluidigm
19	CD200	OX-104	Fluidigm
20	CD25	2A3	Fluidigm
21	CD27	L128	Fluidigm
22	CD28	CD28.2	Fluidigm
23	CD3	UCHT1	Fluidigm
24	CD33	WM53	Fluidigm
25	CD34	581	Fluidigm
26	CD38	HIT2	Fluidigm
27	CD4	RPA-T4	Fluidigm
28	CD45	HI30	Fluidigm
29	CD45RO	UCHL1	Fluidigm

	Antibody	Clone	Supplier
30	CD56	HCD56	Biologend
31	CD69	FN50	Fluidigm
32	CD8	RPA-T8	Fluidigm
33	CD80	2D10.4	Fluidigm
34	CD86	IT2.2	Fluidigm
35	CD95	DX2	Fluidigm
36	CTLA4	14D3	Fluidigm
37	CXCR4	12G5	Fluidigm
38	CXCR5	RF8132	Fluidigm
39	DNAM-1	TX25	Biologend
40	EOMES	WD1928	Ebioscience
41	FOXP3	PCH101	Fluidigm
42	Gata3	TWAJ	Fluidigm
43	Granzyme	GB11	Fluidigm
44	HLADR	L243	Fluidigm
45	HVEM	122	Biologend
46	ICOS	C398.4A	Fluidigm
47	Ki67	Ki-67	Fluidigm
48	LAG3	11C3C65	Fluidigm
49	NKG2D	OW72	Fluidigm
50	PD-L1	CD274	Fluidigm
51	PD1	EH12.2H7	Fluidigm
52	SLAMF7	162.1	Biologend
53	T-Bet	4B10	Fluidigm
54	TCF1	7F11A10	Biologend
55	TIGIT	MBSA43	Fluidigm
56	TIM3	F38-2E2	Fluidigm
57	VEGFR2	7D4-6	Fluidigm

Table S3. Quality measures of scRNAseq

Cell Type	Median Genes/Cell	Median UMI/Cell	Total Cells
T cell	978	2985	23891
B cell	914	2716	6721
Myeloid cell	1220.5	3719	5864
pDC	1901	6028	463
NK cell	1029	2411	5667
All cells	993	2894	42606

Table S4. Highly Differentially Expressed Genes in Each Cluster

Cluster	Gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj
T1	LTB	0	0.91670112	0.929	0.455	0
	LDHB	0	0.89570163	0.878	0.46	0
	IL7R	0	0.85019827	0.683	0.2	0
	NOSIP	0	0.7984456	0.641	0.252	0
	CCR7	0	0.69293816	0.445	0.108	0
T2	TNFAIP3	0	1.47226662	0.672	0.145	0
	NR4A2	0	1.34552898	0.567	0.145	0
	JUNB	0	1.28180243	0.924	0.659	0
	TSPYL2	0	1.17838369	0.506	0.139	0
	PMAIP1	0	1.17048015	0.396	0.165	0
T3	GZMK	0	2.01806253	0.743	0.069	0
	DUSP2	0	1.44681637	0.706	0.196	0
	RGS1	0	1.2361968	0.346	0.105	0
	CCL5	0	1.2075621	0.919	0.305	0
	IL32	0	1.18695249	0.947	0.428	0
T4	GZMH	0	2.22063019	0.932	0.101	0
	FGFBP2	0	1.38394973	0.742	0.104	0
	CST7	0	1.33884052	0.923	0.23	0
	GZMA	0	1.20537669	0.883	0.222	0
	CTSW	0	1.13052979	0.84	0.269	0
T5	MALAT1	0	0.99344512	1	0.993	0
	FTL	0	-1.1938078	0.584	0.937	0
	OAZ1	2.59E-300	-0.8667835	0.331	0.804	5.30E-296
	GAPDH	5.11E-241	-0.8839908	0.381	0.783	1.05E-236
	CLIC1	1.00E-230	-0.7877622	0.125	0.548	2.05E-226
T6	IFI44L	4.71E-137	0.89033086	0.332	0.042	9.63E-133
	CD52	5.22E-50	0.65081849	0.94	0.704	1.07E-45
	IFITM1	1.19E-35	0.69539883	0.608	0.299	2.44E-31
	FLT3LG	7.36E-33	0.40774442	0.352	0.121	1.51E-28
	B2M	1.65E-31	0.31674443	1	0.986	3.38E-27
NK1	GNLY	0	2.34562861	0.973	0.256	0
	GZMB	0	2.24477348	0.937	0.146	0
	PRF1	0	2.21174003	0.9	0.144	0
	NKG7	0	2.08441301	0.994	0.339	0
	SPON2	0	2.02052901	0.76	0.078	0
NK2	CMC1	0	1.88307361	0.884	0.214	0
	XCL1	0	1.80742181	0.605	0.03	0
	XCL2	0	1.77593293	0.627	0.056	0
	KLRB1	0	1.70004243	0.846	0.203	0
	KLRC1	0.00E+00	1.6911337	0.527	0.036	0.00E+00
NK3	CCL3	0	2.28727795	0.54	0.099	0
	IFIT2	0.00E+00	1.81494611	0.372	0.045	0.00E+00
	EIF1	3.62E-297	1.01155107	0.999	0.955	7.41E-293

	SERTAD1	5.58E-289	1.20105923	0.409	0.07	1.14E-284
	H3F3B	3.64E-259	0.93357441	0.996	0.922	7.44E-255
M1	S100A8	0	4.86452505	0.986	0.187	0
	S100A9	0	4.76171825	0.99	0.19	0
	LYZ	0	4.10131896	0.998	0.154	0
	S100A12	0	3.5609141	0.868	0.032	0
	FCN1	0	2.79458947	0.917	0.046	0
M2	CST3	0	2.36702179	1	0.267	0
	HLA-DRB1	0	2.14617117	0.999	0.354	0
	FCER1A	0	2.09638709	0.866	0.019	0
	CLEC10A	0	1.54289516	0.697	0.006	0
	LGALS1	0.00E+00	1.37982972	0.996	0.382	0.00E+00
M3	LST1	0	2.86890711	0.997	0.183	0
	AIF1	0	2.48419896	0.997	0.209	0
	FCGR3A	0	2.27979303	0.968	0.118	0
	COTL1	0	2.16586742	0.985	0.361	0
	SAT1	0	2.16339283	0.995	0.432	0
pDC	IRF8	0	2.46588222	0.946	0.1	0
	IRF7	0	2.19424903	0.922	0.094	0
	PLD4	0	2.13183986	0.948	0.055	0
	LILRA4	0	2.07313462	0.881	0.009	0
	ITM2C	0	2.07016956	0.955	0.15	0
B1	CD79A	0	2.22383476	0.882	0.054	0
	MS4A1	0	1.99457801	0.749	0.033	0
	CD74	0	1.98972013	0.999	0.639	0
	HLA-DRA	0	1.84891443	0.992	0.318	0
	LINC00926	0	1.58712633	0.594	0.009	0
B2	CD79B	0	2.71596402	0.976	0.195	0
	TCL1A	0	2.56900877	0.717	0.06	0
	VPREB3	0	2.22157959	0.814	0.067	0
	VPREB1	0	2.16787949	0.569	0.01	0
	SOX4	0	2.08853404	0.817	0.075	0