Supplemental Methods

Analysis of single cell RNA-Sequencing (scRNA-seq) Data

scRNA-seq data from BM samples of patients with MM (GSE186448)[1] were analysed using the scflow pipeline (https://github.com/cribbslab/scflow). The kallisto/bustools workflow[2] was used to pseudoalign the reads, using a kmer size of 31 base pairs to the GRCh38 (hg38) reference transcriptome. The output was converted to a singleCellExperiment class object for initial filtering and then converted to a Seurat object for further downstream processing. Quality control and filtering was performed using a mitochondrial ratio cuttoff of 0.1, fewer than 500 UMIs, or fewer than 300 or greater than 6000 features were removed. Clustering and sample integration were performed using Seurat (v4)[3]. Automated cell type annotation using singleR[4], scCLassify[5] and clustifyr[6] were used as a guide for manual annotation. Seurat's Wilcoxon approach was used for differential gene expression analysis.

Published scRNA-seq and associated CITE-seq data from two healthy control BM samples, obtained with donor consent from AllCells (GSE139369)[7] were analysed using scanpy 1.7.0[8]. CD3 protein-expressing bone marrow single-cells were processed to 7 clusters. Briefly, size factor-normalised log-transformed scaled highly variable genes were used to calculate principal components for neighbourhood graph construction, in turn used for leiden clustering[9] and UMAP calculation[10]. T cell phenotype was assigned to each cluster using canonical marker genes and surface expression.

Table S1: General and Clinical Characteristics of Patients and Controls

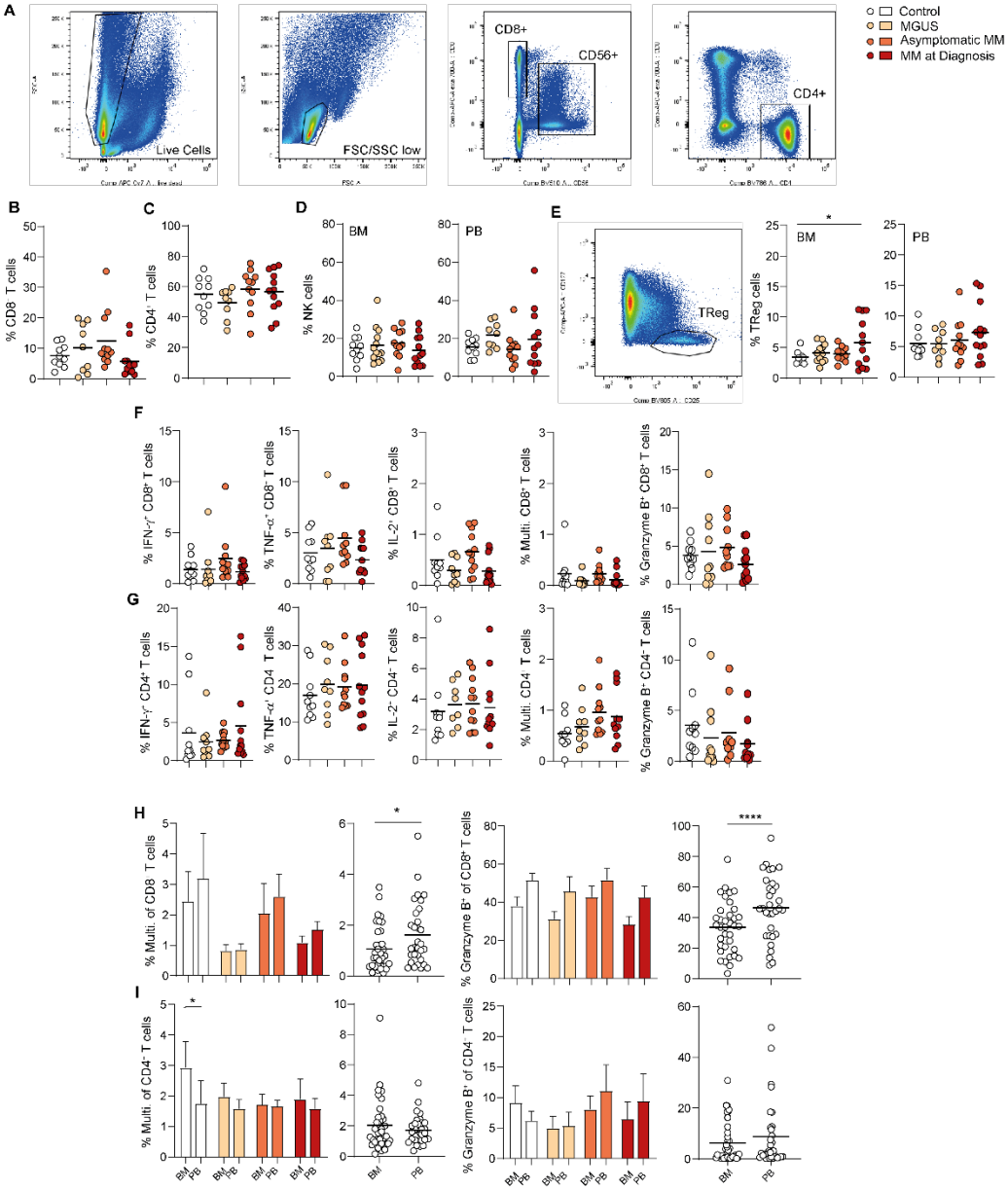
	Control	MGUS	Asymptomatic MM	MM at Diagnosis	MM on relapse	MM in remission
n	10	13	11	12	14	12
Age – Median (Range)	83 (44-97)	65 (50-91)	72 (56-82)	74 (48-86)	65 (47-88)	73 (53-86)
Sex - % male	30	46	50	50	50	60
MMPC Abundance						
Trephine % - Median (Range)	N/A	5 (0-9)	16 (11-50)	55 (15-70)	40 (0-80)	5 (0-8)
Paraprotein (PP) Abundance and Type						
PP level - Median (Range)	N/A	10.5 (2.0-16.0)	20.5 (6.8-26.0)	21.0 (0.0-51.6)	14.0 (0.0-17.0)	0.0 (0.0-4.0)
IgG (%)	N/A	66.7	85.7	88.9	92.8	58.3
IgA (%)	N/A	33.3	14.3	11.1	0	41.7
Light Chain (%)	N/A	0	0	0	7.2	0

Table S2: Treatment details for MM patients on relapse or in remission

	MM on relapse	MM in remission
Lines of therapy – Mean (Range)	1.7 (1-3)	1.3 (1-3)
% Autologous stem-cell transplant	57.1	33.3
Treatment at Sampling		
% Immunomodulatory Drug	50.0	91.7
% Proteasome Inhibitor	78.6	66.6
% Glucocorticoid	92.9	83.3
% Alkylating Agent	50.0	0.0
% CD38-targeting agent	28.6	41.6

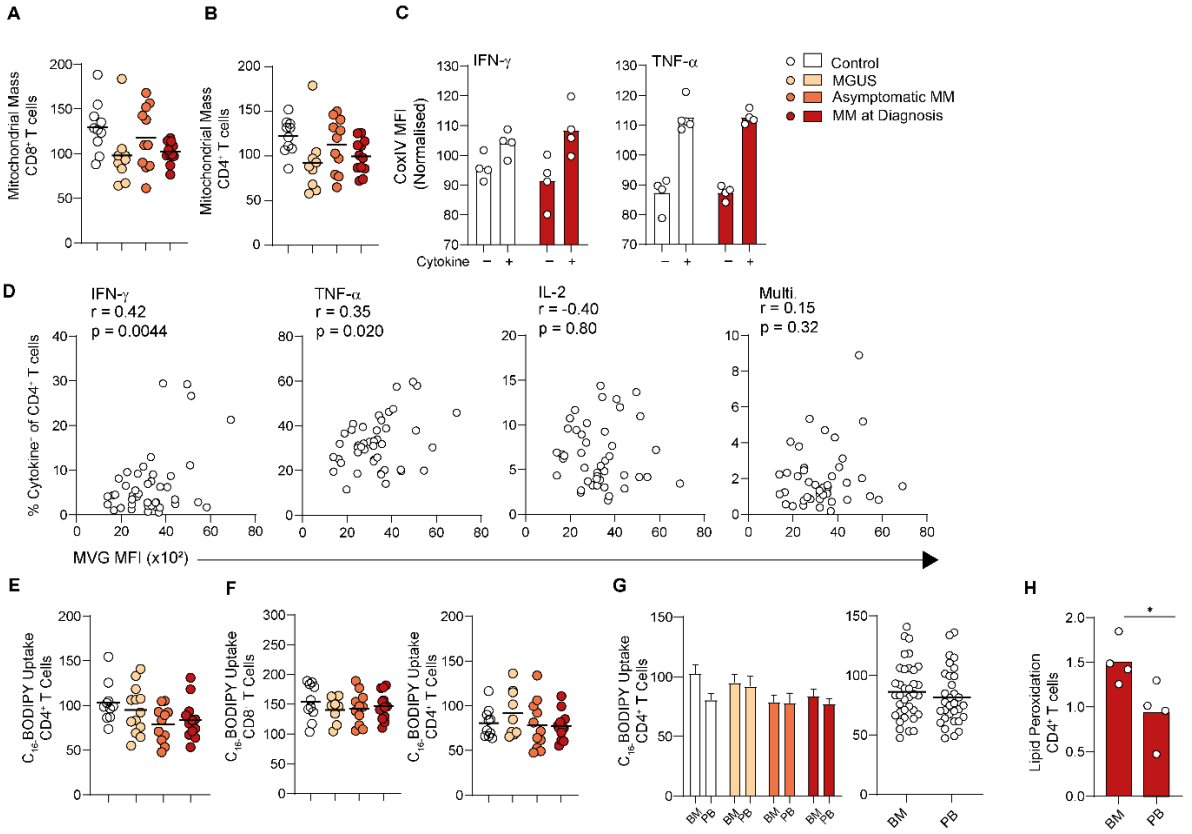
Table S3: Details of flow cytometry antibodies

Antibody	Clone	Supplier and Cat #
Anti-CD4 BV785	OKT4	Biolegend #317442
Anti-CD8 AF700	SK1	Biolegend #344724
Anti-CD56 BV510	HDC56	Biolegend #318340
Anti-CD127 APC	A019D5	Biolegend #351342
Anti-CD25 BV605	BC96	Biolegend #302632
Anti-CD45RA BV421	HI100	Biolegend #304130
Anti-CCR7 BUV373	2-L1-A	BD #749676
Anti -TIGIT BUV395	741182	BD #747845
Anti-PD1 PE-cy7	EH12.2H7	Biolegend #329918
Anti-CD36 PE	TR9	Molecular probes A15777
Anti-IFN-γ FITC	B27	Biolegend #506504
Anti-TNF-α PE	MAb11	Biolegend #502909
Anti-IL-2 BV605	1-17H12	Biolegend #500332
Anti-Granzyme B	QA16A02	Biolegend #372212
Anti-COX IV AF647	mAbcam339851	Abcam ab197491
Anti-VDAC unconjugated	2OB12AF2	Abcam ab14734
Goat-anti-mouse PE-cy7	Poly4053	Biolegend #405315



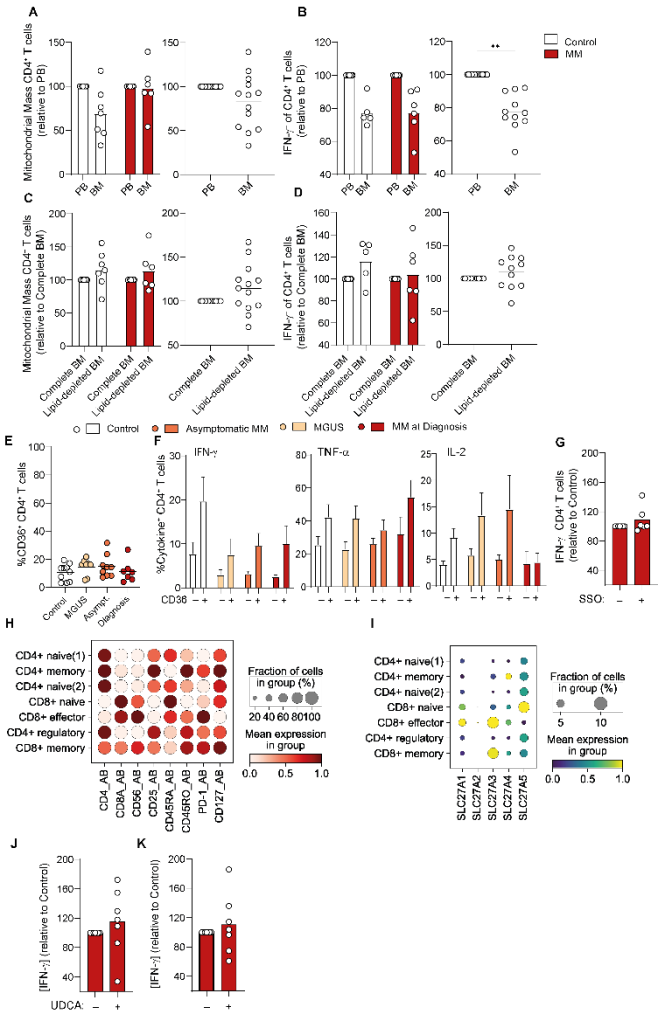
Supplementary Figure 1:

(**A**) Representative dot plots show cell population gates for $CD4^+$ and $CD8^+$ T cells, and $CD56^+$ NK cells within BM and PB live FSC/SSC low cells. (**B-C**) Graphs show percentage of (**B**) $CD8^+$ and (**C**) $CD4^+$ T cells in PB mononuclear cells from controls (n=10), individuals with MGUS (n=13), asymptomatic MM (Asympt. n=11) or MM at diagnosis (Diagnosis n=12). (**D**) Proportions of $CD56^+$ NK cells in BM and PB mononuclear cells from each group as indicated. (**E**) Representative dot plots show cell population gate for $CD4^+CD127^-CD25^+$ TReg cells. Proportion of $CD4^+CD127^-CD25^+$ TReg cells in BM and PB mononuclear cells from each group as indicated. (**F-G**) Proportion of PB FSC/SSC low cells from indicated groups expressing (**F**) CD8 or (**G**) CD4 and IFN-γ, TNF-α, IL-2, all three cytokines (Multifunctional) or Granzyme B as indicated, after 4 hours stimulation via CD3/CD28 in presence of brefeldin A. (**H-I**) Proportion of (**H**) $CD8^+$ and (**I**) $CD4^+$ T cells within BM or PB mononuclear cells from indicated groups expressing IFN-γ, TNF-α and IL-2 (Multifunctional) or Granzyme B after 4 hours stimulation as in (F-G). Proportions within the BM and PB are directly compared for each group individually and for all patients combined. Significance was calculated using (B-G) one-way or (H-I bars) two-way ANOVA and Sidak's multiple comparison tests or (H-I circles) paired t test; *p<0.05, **p < 0.01, ***p < 0.0001.



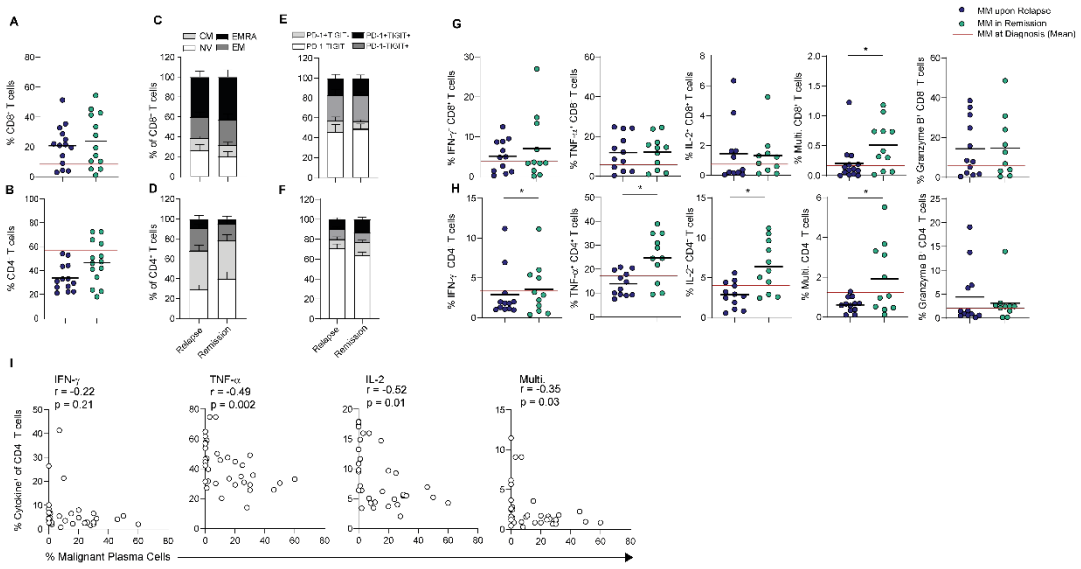
Supplementary Figure 2:

(**A-B**) Mitochondrial mass of (**A**) $CD8^+$ and (**B**) $CD4^+$ T cells PB mononuclear cells from controls (n=10), individuals with MGUS (n=13), asymptomatic MM (Asympt. n=11) or MM at diagnosis (Diagnosis n=12) assessed using mitoview green. (**C**) CoxIV MFI of $CD4^+$ T cells positive or negative for IFN-γ or TNF-α as indicated from control (n=4) or MM at diagnosis (n=4) samples. (**D**) Correlation of mitochondrial mass (MVG fluorescence) with frequency of BM $CD4^+$ T cells positive (+) for the indicated cytokines for all patient samples combined. (**E-F**) C_{16}-BODIPY uptake of (**E**) BM $CD4^+$ T cells, (**F**) PB $CD8^+$ and PB $CD4^+$ T cells within indicated groups. (**G**) C_{16}-BODIPY uptake of BM and PB $CD4^+$ T cells are directly compared for each group and for all patient samples combined. (**H**) Lipid peroxidation capacity of $CD4^+$ T cells within BM and PB mononuclear cells from MM patients at diagnosis (n=4), assessed using the lipid peroxidation probe Bodipy 581/591. Significance was calculated using (A-B, E-F) one-way or (C, G bars) two-way ANOVA and Sidak's multiple comparisons test, (D) Pearson's correlation (G circles, H) paired t test, *p<0.05, **p < 0.01, ***p < 0.0001.



Supplementary Figure 3:

(**A-B**) BM mononuclear cells from controls (n=6) or relapsed MM patients (n=6) were cultured in autologous PB or BM plasma as indicated for 72 hours. (**A**) $CD4^+$ T cell mitochondrial mass was assessed by flow cytometry using mitoview green and is shown for each group (left) and both combined (right). (**B**) At 72 hours T cells were activated with PMA/ionomycin and expression of IFN-γ was assessed within $CD4^+$ T cells by flow cytometry. (**C-D**) BM mononuclear cells were cultured and assessed as in (A-B) but in either complete or lipid-depleted BM autologous plasma as indicated. (**E**) Proportion of $CD4^+$ T cells expressing CD36 within controls (n=10), individuals with MGUS (n=8), asymptomatic MM (Asympt. n=9) or MM at diagnosis (Diagnosis n=7) were assessed by flow cytometry. (**F**) Expression of IFN-γ, TNF-α and IL-2 by CD36 positive and negative $CD4^+$ T cells within indicated BM mononuclear cell samples. (**G**) BM mononuclear cells from MM patients at diagnosis (n=7) were cultured in autologous BM plasma as in (A-D), in absence or presence of the CD36 inhibitor, SSO and assessed for IFN-γ expression within $CD4^+$ T cells by flow cytometry. (**H-I**) Dot plots demonstrating (**H**) phenotypic T cell cluster allocation by indicated protein expression and (**I**) SLC27A1-5 gene expression within indicated T cell clusters from CITE-Seq data of two healthy control BM samples. (**J-K**) BM mononuclear cells from MM patients at diagnosis (n=7) were cultured in autologous PB (**J**) or BM (**K**) plasma as in (A-D), in absence or presence of the FATP5 inhibitor UDCA and assessed for IFN-γ expression by ELISA. Significance was calculated using (A-D circles, G, J-K) Wilcoxon matched-pairs signed rank test; $^{**}p < 0.01$, $^{***}p < 0.0001$.



Supplementary Figure 4:

(**A-B**) Proportion of (**A**) $CD8^+$ and (**B**) $CD4^+$ T cells within PB mononuclear cells from relapsed MM patients (n=14) and MM patients in remission (n=12). (**C-D**) Proportions of NV, CM, EM and EMRA (**C**) $CD8^+$ and (**D**) $CD4^+$ T cells in indicated PB samples. (**E-F**) Percentage of PD-1^-TIGIT^-, PD-1^+TIGIT^-, PD-1^- TIGIT^+ and PD-1^+TIGIT^+ (**E**) $CD8^+$ and (**F**) $CD4^+$ T cells in PB mononuclear cells from indicated samples. (**G-H**) Proportion of (**G**) $CD8^+$ and (**H**) $CD4^+$ T cells within PB mononuclear cells from each group expressing IFN-γ, TNF-α, IL-2, all three of these cytokines (Multifunctional) or Granzyme B after 4 hours of stimulation via CD3/CD28 brefeldin A. (**I**) Correlation of percentage of malignant plasma cells within the BM sample with frequency of BM $CD4^+$ T cells positive (+) for the indicated cytokines for samples combined for patients with MM at diagnosis (n=12), relapsed MM patients (n=14) and MM patients in remission (n=12). Significance was calculated using (A-B, G-H) unpaired t test and (I) Pearson correlation; *p<0.05.

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