**SUPPLEMENTAL METHODS**

## Patient bone marrow biopsies

Briefly, 3-mm trephine-needle bone marrow biopsies were obtained from the iliac crest of 18 controls, 16 MGUS subjects, and 19 newly-diagnosed MM patients before and after treatment with bortezomib /cyclophosphamide-dexamethasone, followed by autologous stem cell transplantation. All included subjects and patients were age and sex-matched and the mean age for all groups was 57-59 years. The control cohort originated from patients undergoing a hematological examination, showing no skeletal disorder or treatment known to affect the bone. The mean time between harvest of the diagnostic biopsy and the response evaluating biopsy was 186 (98–300) days, while the mean time between the date of stem cell infusion and the biopsy sampling was 58 (19–117) days. These samples have been previously described (1).

***Histomorphometry of patient bone marrow biopsies- additional details***

Stained sections were scanned at x20 using a Hamamatsu NanoZoomer 2.0 HT Digital slide scanner. Light microscopic analysis of the bone marrow biopsies was performed using NanoZoomer Digital Pathology view2 (NDP.view2) software with superimposed point- and box-grids. Masson’s trichrome stained sections were used for the assessment of adipocytes, while CD138 immunostained sections were used to assess tumor load.

## Animal Experiments- additional details

For the SCID-Beige MM.1Sgfp+luc+ myeloma xenograft model, twelve-week old female Fox Chase SCID-Beige mice (Charles River Laboratory; Wilmington, MA) were housed in the SPF facility at Maine Medical Center Research Institute. Upon arrival, SCID-Beige mice were group housed (n=2-5) with Alpha-Dri Plus Bedding (Shepherd Specialty Paper, TN) and had *ad libitum* access to food (TEKLAD Global 2919 irradiated diet, ENVIGO, IN, USA) and autoclaved water in their home cages for the duration of the experiment. Naïve mice were randomly allocated to either group (naïve/vehicle or MM.1S) prior to tumor cell injection on day 0. C57BL/KaLwRijHsd (BKAL) (Harland, Netherlands) mice were bred and raised in the SPF Australian BioResources Facility at The Garvan Institute of Medical Research. BKAL mice (n=2-5) were housed in Makrolon® polycarbonate transparent or Udel® polysulphone amber cages and lids with ad libitum access to food and water for the duration of the experiments. Six- to eight-week old naïve BKAL female mice were randomly assigned to either naïve, or tumor cell receiving cohorts at day 0 (day of injection) at the Biological Testing Facility at the Garvan Institute. Group sizes were selected based on previous experiment within each model system.

## Histological analysis of mouse samples

Mouse femurs were fixed in 10% neutral buffered formalin overnight and decalcified for 3-5 weeks in 20% EDTA (pH 8.0) solution. Bones were then paraffin embedded, sectioned (5 µm), and stained with hematoxylin and eosin (Richard Allen Scientific, Canton, MI), following manufacturer’s instructions. SCID-Beige histology images were taken using a Nikon Eclipse 80i microscope, while C57BL/KaLwRiJHsd images were captured by Scanscope CS2, with images generated by Aperio Imagescope v11.2.0.780. Adipocytes were quantified utilizing ImageJ software as previously described (2).

***Quantitative PCR***

For qPCR, cDNA synthesis was completed using Multiscribe reverse transcriptase (High Capacity cDNA, Thermo Fisher Scientific) per manufacturer’s instructions. Relative transcript expression was determined using SYBR Master Mix (Bio-Rad, Hercules, CA) by performing qPCR reactions with a CFX-96 thermocycler (Bio-Rad). Target transcripts were normalized to β-actin using the 2-ΔΔct method. Data were analyzed using Bio-Rad CFX Manager 3.1 and Microsoft Excel.

## Primers utilized in qPCR experiments

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| **Gene Target (Symbol)** | **Species** | **Forward Primer (5’ à3’)** | **Reverse Primer (5’ à3’)** |
| Beta Actin (*Actb*) | Mouse | CTCTGGCTCCTAGCACCATGAAGA | GTAAAACGCAGCTCAGTAACAGTCCG |
| Peroxisome proliferator activated receptor gamma (*Pparg*) | Mouse | GCCCACCAACTTCGGAATC | TGCGAGTGGTCTTCCATCAC |
| Adiponectin (*Adipoq*) | Mouse | TGTTCCTCTTAATCCTGCCCA | CCAACCTGCACAAGTTCCCTT |
| Preadipocyte factor 1 (*Pref1*) | Mouse | CTAACCCATGCGAGAACGAT | GCTTGCACAGACACTCGAAG |
| CCAAT enhancer-binding protein alpha (*Cebpa*) | Mouse | TGGACAAGAACAGCAACGAG | TCACTGGTCAACTCCAGCAC |
| Leptin (*Lep*) | Mouse | ACCCCATTCTGAGTTTGTCC | TCCAGGTCATTGGCTATCTG |
| Fatty acid binding protein 4 (*Fabp4*) | Mouse | gctgcagcctttctcacc | cactttccttgtggcaaagc |
| Beta Actin (*ACTB*) | Human | GGACCTGACTGACTACCTC | GCCATCTCTTGCTCGAAG |
| Peroxisome proliferator activated receptor gamma (*PPARG*) | Human | GCTTCTGGATTTCACTATGG | AAACCTGATGGCATTATGAG |
| CCAAT enhancer-binding protein alpha (*CEBPA*) | Human | ACTGGGACCCTCAGCCTTG | TGGACTGATCGTGCTTCGTG |
| Fatty acid binding protein 4 (*FABP4*) | Human | AACCTTAGATGGGGGTGTCC | TGGTTGATTTTCCATCCCAT |
| Interleukin 6 (*IL6*) | Human | CCGGGAACGAAAGAGAAGCT | GCGCTTGTGGAGAAGGAGTT |
| Colony stimulating factor 2 (*CSF2*) | Human | GGCCCCTTGACCATGATG | TCTGGGTTGCACAGGAAGTTT |
| Chemokine (C-X-C motif) ligand 1 (*CXCL1*) | Human | GAAAGCTTGCCTCAATCCTG | CACCAGTGAGCTTCCTCCTC |
| Chemokine (C-X-C motif) ligand 2 (*CXCL2*) | Human | AACTGCGCTGCCAGTGCT | CCCATTCTTGAGTGTGGCTA |
| Interleukin 6 (*Il6*) | Mouse | TCCTCTCTGCAAGAGACTTCC | GGAGAGCATTGGAAATTGGGG |
| Chemokine (C-X-C motif) ligand 1 (*Cxcl1*) | Mouse | CCGAAGTCATAGCCACACTCA | CTCCCACACATGTCCTCACC |
| Chemokine (C-X-C motif) ligand 2 (*Cxcl2*) | Mouse | CAGGCTACAGGGGCTGTTGT | ACATCAGGTACGATCCAGGC |
| FK506 binding protein 51 (*FKBP5*) | Human | AGTTACATCCCCCATGCCAAG | GGGGATTGTCGCTTCGTAGT |
| Kruppel Like Factor 9 (*KLF9*) | Human | CACCGAATCTGGGTCGAGTC | GCCGTTCACCTGTATGCACT |
| Poly(ADP-Ribose) Polymerase Family Member 9 (*PARP9*) | Human | CCGGAAGTGGGCCACCATATC | CGGCCACCATGGAAAAGTCC |

## Microarray gene expression analysis- Continued

Ribolock (Thermo Fisher Scientific, Waltham, MA) was added to inhibit RNA degradation in samples processed for microarray at a final concentration of 1U/µL. Differential expression of functional groups was assessed through Pathway-ANOVA and GO-ANOVA analyses in Partek Genomic Suite, which utilized KEGG Pathway and GO term databases, respectively. Normalized gene expression values were also subjected to a gene set enrichment analysis (GSEA) using the Java implementation from the Broad Institute (6). Several Molecular Signatures Database (MSigDB v6.1, Dec 2, 2017) collections were used to identify gene sets significantly enriched in MM.1S vs control cultures, including H (Hallmark), C2 (curated gene sets) and C5 (Gene Ontology, GO, gene sets), which contain 50, 3689, and 4429 gene sets respectively, when gene set size filters min=15 and max=500 were applied (7). Only those gene sets with a false discovery rate (FDR) < 25% were considered significantly enriched.

## RNA-Sequencing of human BMAT and MM cell co-cultures- Continued

All downstream analyses were completed utilizing an expression cutoff of 0.3 RPKM. For differentially expressed genes, relative fold change of >2.0 for co-cultured samples vs. their control was used for downstream analyses. Analysis of MM-adipocytes compared to control adipocytes utilized an additional layer of stringency (p<0.05 by Student’s T-test), for the selection of up- and down-regulated genes. Gene networks were compared utilizing STRING v11 (ELIXIR; Hinxton, UK) (8) and GeneMania (9). Common and unique gene expression changes were compared and visualized using FunRich v3.1.3 (10). Supervised analysis of genes encoding senescence associated secretory proteins, described in (11), and those involved in adipogenesis (KEGG Pathway Database; Kyoto, Japan) via expression values and hierarchical clustering was performed with matrix visualization and MORPHEUS analysis software (<https://software.broadinstitute.org/morpheus>).

***Protein assessment in adipocyte conditioned media- additional details***

Array membranes were imaged using the ChemiDoc Touch Imaging System (Bio-Rad), and relative protein levels (MM-adipocyte versus adipocyte) were determined utilizing Image Lab 6.0 Software (Bio-Rad).

## Mitochondria functional analysis - Continued

Briefly, medium was changed to Seahorse XF Base Medium Minimal DMEM without phenol red supplemented with 1 mM pyruvate, 2 mM glutamine, and 25 mM glucose, pH 7.4, and the cells were equilibrated for 1 h at 37°C in a CO2-free incubator. After measurements of basal respiration rate, cells were treated sequentially with oligomycin (1.25 μM final concentration), FCCP (1 μM), and a combination of antimycin (0.5 uM)/rotenone (0.5 μM)/20mM Hoechst Dye and respiration rates after each treatment were measured. Post assay cells were lysed and total protein content per well was measured using a standard BSA assay. The resulting protein values were then used to normalize the data per well. OCR and ECAR were calculated by the Seahorse XFe96 software, Wave version 2.6. Seahorse experiments were run at least three different times with an n=46 wells/treatment. Depicted within is a representative run of these experiments.

## New analyses of previously published datasets

Publicly available microarray data from 160 clinical patient primary plasma cell samples (GSE6477; Chng et al. 2007) were downloaded via GEO. Expression data were log transformed prior to an ANOVA to compare expression between healthy plasma cells and plasma cells extracted from patients in different stages of myeloma disease progression. Estimated means and effect relative to normal were calculated for each MM disease stage, yielding approximately 35% differentially expressed genes captured in the array. These differentially expressed genes were then cross-referenced with a gene list derived from GO terms relating to adipogenesis (see below).

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| **GO Term** |
| White fat cell differentiation involved in mammary gland fat development |
| White fat cell differentiation |
| Leptin-mediated signaling pathway |
| Fat cell differentiation |
| Positive regulation of fat cell differentiation |
| Negative regulation of fat cell apoptotic process |
| Regulation of fat cell apoptotic process |
| Fat cell apoptotic process |
| Positive regulation of white fat cell proliferation |
| Negative regulation of white fat cell proliferation |
| Regulation of white fat cell proliferation |
| White fat cell proliferation |
| Response to leptin |
| Regulation of fat cell differentiation |
| Negative regulation of fat cell differentiation |
| Positive regulation of fat cell apoptotic process |

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