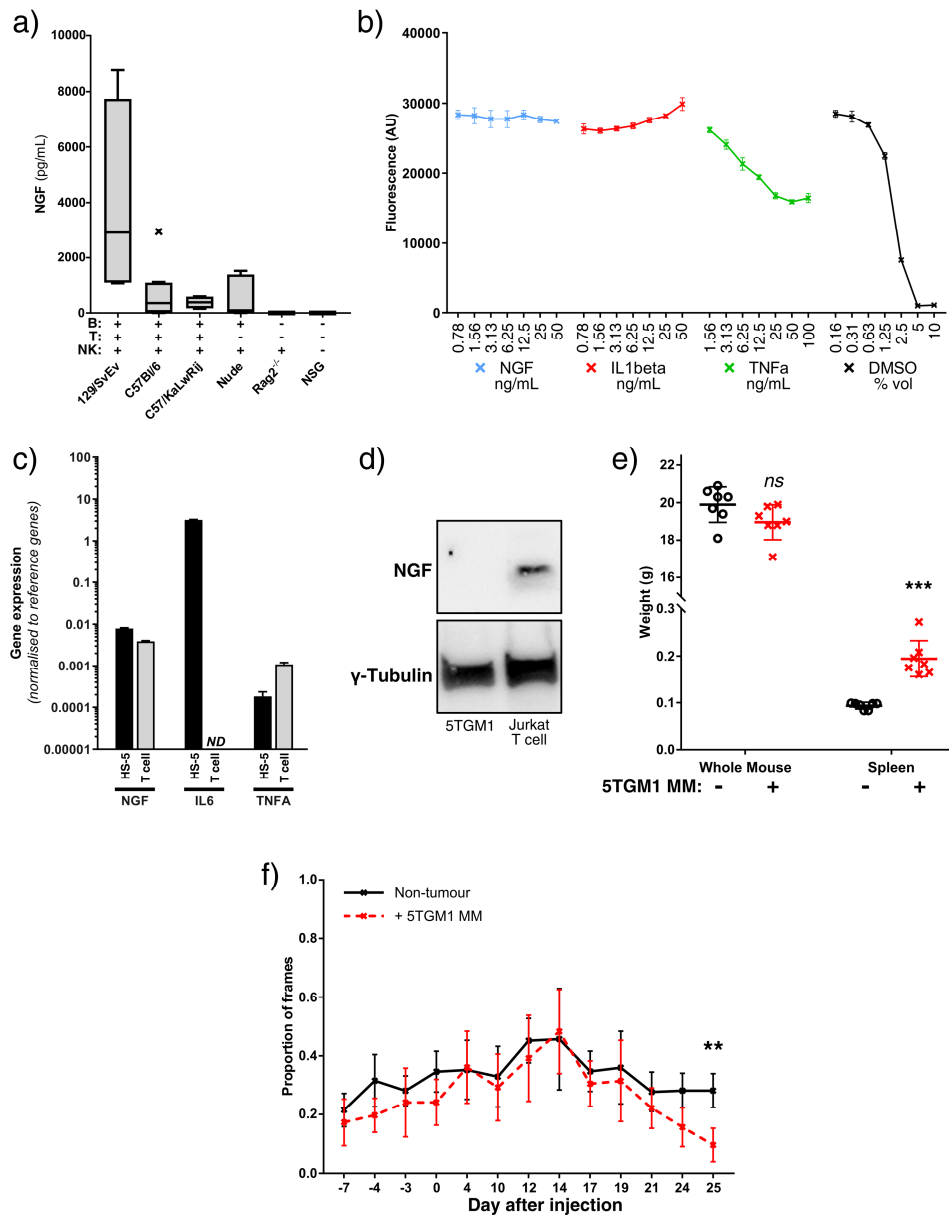


**Multiple myeloma increases nerve growth factor and other pain-related markers through interactions with the bone microenvironment:**

**Supplementary Information**

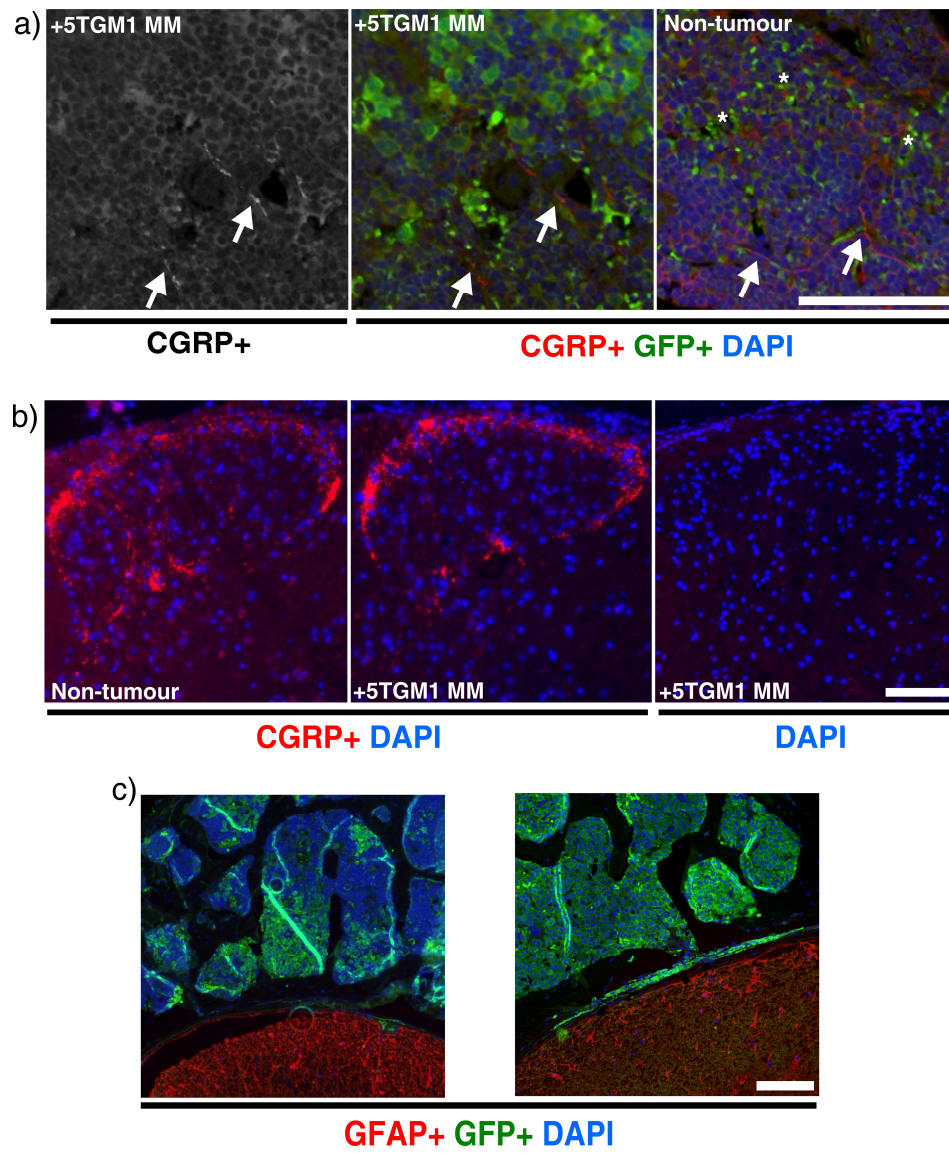
Sam W. Z. Olechnowicz, Megan M. Weivoda, Seint T. Lwin, Szi K. Leung, Sarah Gooding, Guido Nador, Kassim Javaid, Karthik Ramasamy, Srinivasa R. Rao, James R. Edwards, Claire M. Edwards.

## Supplementary Figures

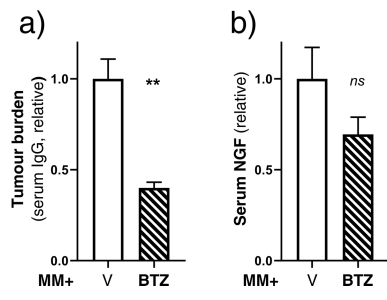


**Figure S1.**





**Figure S2.**



**Figure S3.**

Species	Gene	Forward	Reverse	Amplicon size (bp)
Human	<i>NGF</i>	ggcagaccgcaacattact	caccaccgacctcgaagtc	135
Human	<i>TNFA</i>	cccagggacctctctaatc	ggtttgctacaacatgggctaca	98
Human	<i>NTRK1</i>	aacctcaccatcgtgaagagt	tgaaggagagattcaggcgac	91
Human	<i>NGFR</i>	ggcacctccagaacaagacctc	acagggatgaggttgctcggtg	114
Human	<i>GAPDH</i>	acaactttggtatcgtggaagg	gccatcacgccacagtctc	101
Mouse	<i>NGF</i>	aggcccatggtacaatcccttca	atctccaaccacacactgacact	119
Mouse	<i>TNFA</i>	ttctattcctgcttgaggca	ttgagaagatgatctgagtgtgagg	150
Mouse	<i>NTRK1</i>	ggtctttctcgtgagtgtctac	gctgaaagtcctgccgagcatt	107
Mouse	<i>NGFR</i>	cctgcctggacagtgttacg	cacaggagcggacatactc	102
Mouse	<i>GAPDH</i>	tcaacagcaactcccactcttcca	accctgttgctgtagccgtattca	115
Mouse	<i>POLR2A</i>	gcaccatcaagagagtgcag	gggtattgataccaccctctg	85
Mouse	<i>ADIPOR1</i>	taacctggctgataacgggc	tcaaggcgtggctttgtttg	100
Mouse	<i>ADIPOR2</i>	gccttcctaggccgataac	atgcccaaaggctctcagtc	102

Species	Gene	Catalogue Number	Fluorescent Dye
Human	<i>NGF</i>	Hs00171458_m1	FAM
Human	<i>IL6</i>	Hs00985639_m1	FAM
Human	<i>TNFA</i>	Hs00174128_m1	FAM
Human	<i>GAPDH</i>	Hs99999905_m1	VIC
Human	<i>POLR2A</i>	Hs00172187_m1	VIC
Mouse	<i>NGF</i>	Mm00443039_m1	FAM
Mouse	<i>IL6</i>	Mm00446190_m1	FAM
Mouse	<i>GAPDH</i>	4352339E-1207039	VIC
Mouse	<i>POLR2A</i>	Mm00839493_m1	VIC

**Table S1.**

## Supplementary Figure Legends

**Figure S1.** (a) Tukey plot of serum NGF levels in mouse strains as indicated, under control conditions. Presence or lack of B, T and natural killer (NK) immune cells in each strain is indicated. (b) 5TGM1 cells were grown in 5% FBS RPMI with recombinant cytokines concentrations as indicated for 72 hours, then with resazurin for 4 hours to measure cell activity by fluorescence. DMSO is included as a positive control for cell death. (c) Gene expression quantified by Taqman qPCR for *NGF*, *IL6* and *TNFA* transcripts in human HS-5 stromal cells and Jurkat T cells, relative to the average of reference genes *GAPDH* and *POLR2A*. (d) Western blot of 5TGM1 and Jurkat T cell lysates, probed for NGF and  $\gamma$ -Tubulin. (e) Whole body weight and dissected spleen weights were taken on day 25 after inoculation with 5TGM1 cells compared to non-tumour mice. (f) Quantification of time tumour-bearing and control mice spent running, using AnimApp automated mouse tracking software. Data is shown as proportion of video frames a mouse spent with instantaneous speed above a 7.5px/fr threshold. Statistical comparisons made between “+” and “-” columns; ns: not significant, \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

**Figure S2.** (a) CGRP+ nerves (red channel) detected in MM-bearing and non-tumour KaLwRij long bones, as indicated by arrows. Left panel shows isolated CGRP channel of the centre panel to highlight nerve immunofluorescence. 5TGM1-MM cells are GFP+ (green channel). Examples of non-specific green channel autofluorescence in red blood cells are indicated by asterisks in the non-tumour panel. (b) Representative images of CGRP+ nerves in the dorsal horn of non-tumour and MM-bearing mouse spines, and negative control with no primary antibody. (c) Examples of low burden 5TGM1 MM growth in vertebral body marrow (left panel), and high MM burden leading to a bone lesion with extramedullary growth into spinal canal (right panel), with spinal cord marked by GFAP+. All sections are counterstained with DAPI (blue channel), white scale bars indicate 100 $\mu$ m.

**Figure S3.** C57/KaLwRij strain mice were inoculated with 5TGM1-MM cells, then two weeks later oral bortezomib (BTZ) was administered orally at 0.5mg/kg, repeating three times a week until sacrifice. Serum from cardiac puncture was analysed by ELISA for (a) IgG as a marker of tumour burden and (b) NGF. Statistical comparisons performed by unpaired Student's *t*-test; ns: not significant, \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

**Table S1. Primer sequences or catalogue numbers for RT-PCR and qPCR primers.**

## Full-length gels and blots

Figure 1c):

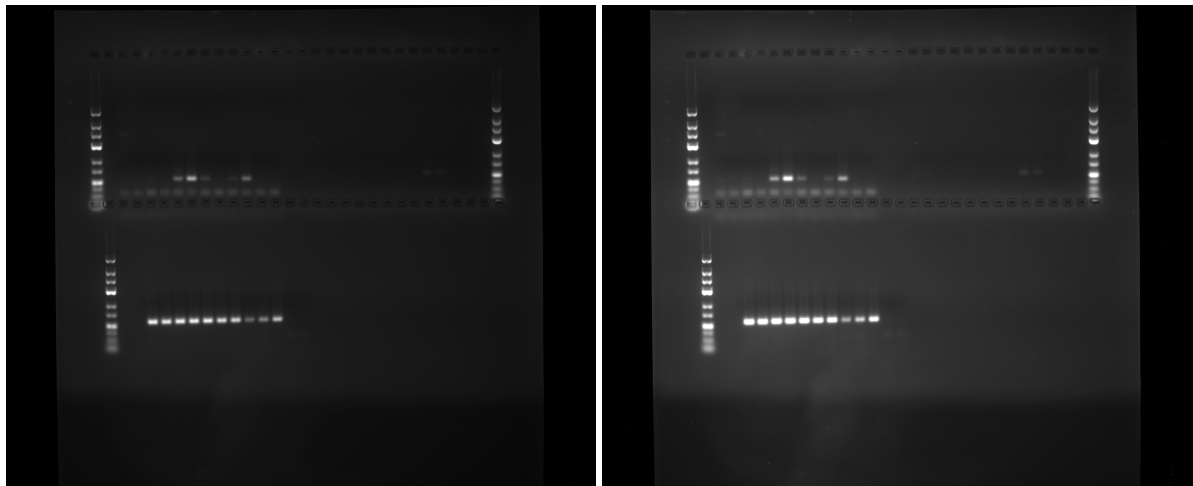
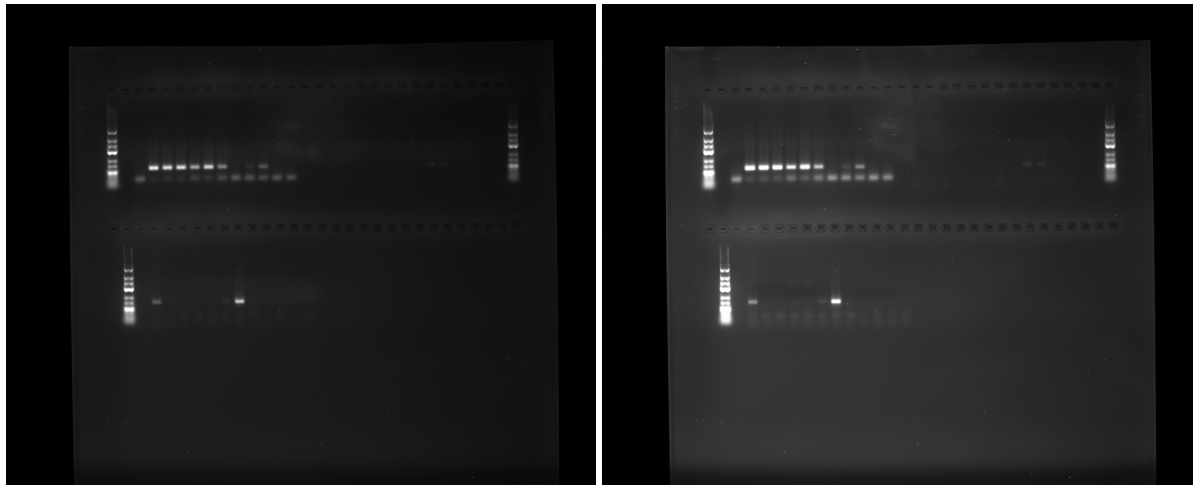


Figure 1d):

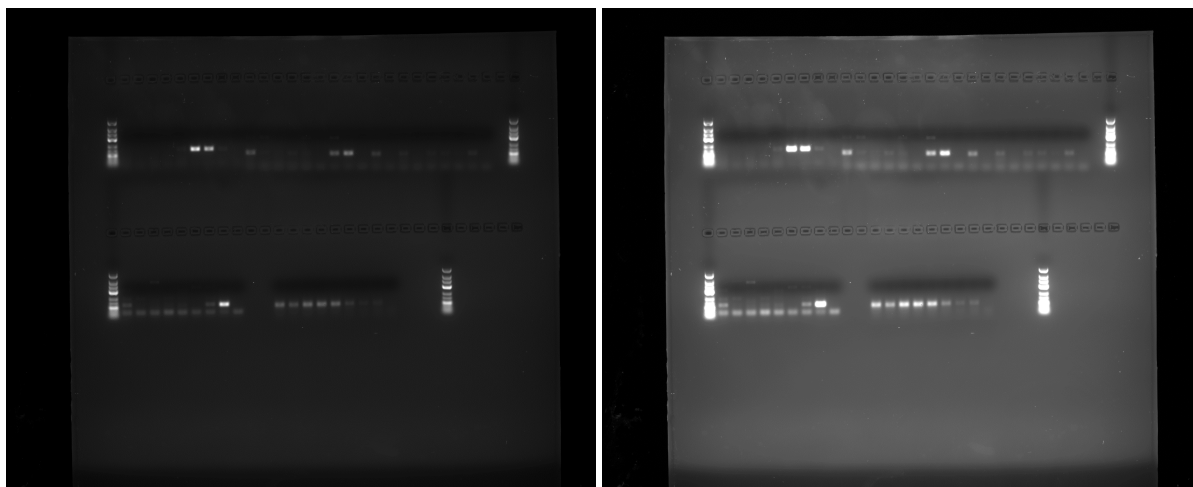


Figure 4b):

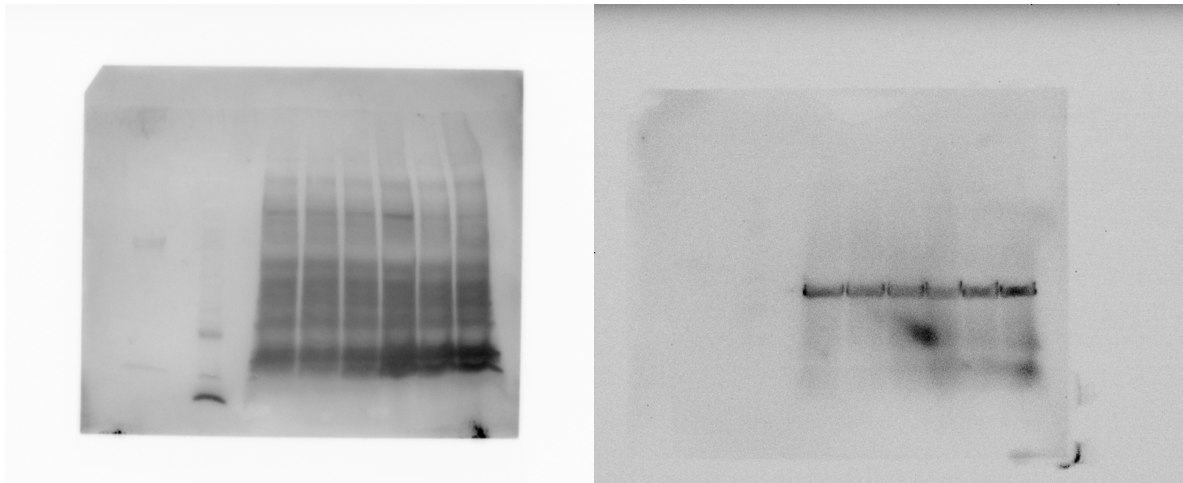


Figure 4c):

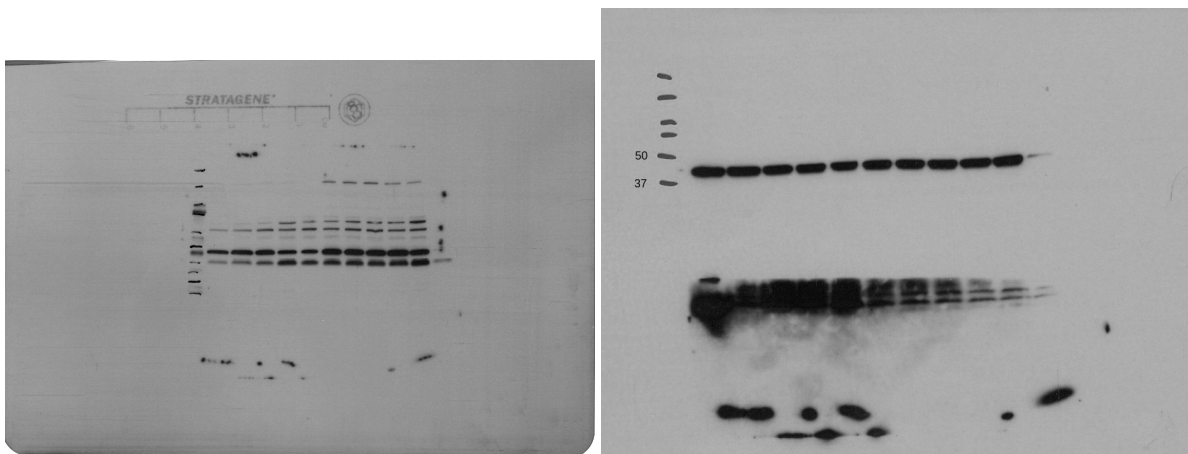


Figure S1d):

