

Supplemental Data

MATERIALS AND METHODS

Western blotting

Total cell lysates were prepared, separated by electrophoresis and transferred to PVDF membranes. Membranes were blocked in TBS plus 5% BSA and 0.1% TWEEN for 1h, before incubating with anti-vimentin (Dako, Glostrup, Denmark), anti-alpha-smooth muscle actin (Dako), anti-fibroblast specific protein 1 (Abcam, Cambridge, MA) or anti- β -actin at 4°C overnight. Antigen-antibody complexes were detected using secondary antibodies conjugated to HRP and visualized by enhanced chemiluminescence (GE Healthcare, Piscataway, NJ).

Flow cytometry

Bone marrow was flushed from the tibia and femur of myeloma-bearing mice. Spleens from myeloma-bearing mice were homogenized in tissue culture media. Cell suspensions were filtered through a 70 μ m filter and then analyzed for GFP using a 3 laser BD LSRII (Becton Dickinson, San Jose, CA).

Fluorescence in situ hybridization

Paraffin-embedded tissue sections from C57Bl6 mice inoculated with ST2 BMSCs were hybridized with mouse IDetect™ Chr Y FISH Paint Probe (Cambio, Cambridge, UK), and counterstained with DAPI according to the manufacturer's instructions. Cells expressing the Y chromosome were scored blind to determine the proportion of positive cells within the bone marrow using MetaMorph (Molecular devices) computer software. 5 fields were assessed per tumor at x40 magnification.

Immunocytochemistry

14M1 BMSCs were fixed in 4% formalin, incubated with anti-FSP-1 (Abcam) and secondary antibody according to manufacturer's instructions. Cells were counterstained with DAPI, and visualized by fluorescence microscopy.

Immunohistochemistry

Tissue sections were dewaxed in xylene, endogenous biotin-blocked (Dako, Glostrup, Denmark), and incubated in 1.5% normal rabbit serum (Vector Laboratories, Peterborough, UK) in 1× TBS (0.05 M Trizma Base, 0.9% NaCl, pH 7.6) for 30 min. Sections were incubated with goat anti-mouse Dkk1 antibody (R&D Systems, Minneapolis, MN, USA), goat IgG isotype control (Sigma Aldrich, St Louis, MO, USA), or normal rabbit serum, diluted 1:6.5 in 1× TBS for 2 h. Antibodies were removed by washing and incubated with biotinylated rabbit anti-goat antibody (Vector Laboratories) for 30 min before incubation with streptavidin-horseradish peroxidase (Vecotr Laboratories) diluted 1:500 in 1× TBS for 40 min. HRP was detected using DAB substrate (Vector Laboratories), and sections were counterstained with Harris's hematoxylin (VWR) before being mounted in DePeX (VWR).