Figure S1

CD138+ cells isolated from either normal BM (n=3) or MM BM (n=6), and MM cell lines (MM.1S, OPM1, OPM2, RPMI.8226, U266, LR7, H929) has been incubated with isotype control or with anti- E, L or P-selectin (10μg/ml) for 1hr followed by FITC-conjugated secondary antibody. Expression of E-, L- and P-selectins has been evaluated using flow cytometry and expressed as ratio between MFI of selectin/MFI of isotype control. Normal plasma cells, MM primary cells, and MM cell lines presented lack of expression of E, L and P-selectins on.

Figure S2

MM1s cells were transfected with either PSGL-1 siRNA or scramble siRNA, and the expression of PSGL-1 has been evaluated using flow cytometry and expressed as ratio between mean fluorescence intensity (MFI) of PSGL-1 and MFI of isotype control. Down-regulation of PSGL-1 by siRNA was detected at the protein level using flow cytometry.

Figure S3

MM1s, RPMI8226, H929 and HUVECs (0.5 x 106 cell/ml) were cultured with increasing concentrations of GMI-1070 (0.1, 0.25, 0.5 and 1 mg/ml) for 24 hours. Cell survival was assessed by measuring 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Chemicon International, Temecula, CA) dye absorbance. GMI-1070 did not have cytotoxic effects on MM cell lines (MM1s, H929 and RPMI8226) and on HUVECs.

Figure S4

HUVECs were transfected with scramble siRNAs or with E (i), L (ii) or P-selectin (iii) siRNA. HUVECs has been incubated with isotype control or with anti- E, L or P-selectin (10μg/ml) for 1hr followed by FITC-conjugated secondary antibody. Expression of E-, L- and P-selectins has been evaluated using flow cytometry and expressed as ratio between MFI of selectin/MFI of isotype control. E and P selectins were downregulated in HUVEC cells, and the absence of expression of L-selectin was not changed..

Figure S5

MM1S cells were co-cultured with stromal cells and treated with GMI-1070 500μM for 24hrs and changes in the mitochondrial potential in response to the Bcl-2 family of proteins were measured by BH3 profiling. BH3 profiling is a functional assay that enables measurement of changes to the Bcl-2 family of proteins regulates the mitochondrial apoptotic pathway (1-3). The BH3-only peptides used sequence and method of synthesis is as previously described(4). The percentage loss of mitochondrial membrane potential is calculated by normalization to the solvent only control DMSO (1%) and the positive control FCCP (100%), and values were expressed as percentile of MM1s cell when cultured alone with no treatment.

1.Cheng, E. H., Wei, M. C., Weiler, et al BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol Cell, 8: 705-711, 2001.

2.Wei, M. C., Zong, W. X., Cheng, E. H., et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science, 292: 727-730, 2001.

3.Ryan, J. A., Brunelle, J. K., and Letai, A. Heightened mitochondrial priming is the basis for apoptotic hypersensitivity of CD4+ CD8+ thymocytes. Proc Natl Acad Sci U S A, 107: 12895-12900, 2010.

4.Certo, M., Del Gaizo Moore, V., Nishino, et al Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. Cancer Cell, 9: 351-365, 2006.

Figure S1

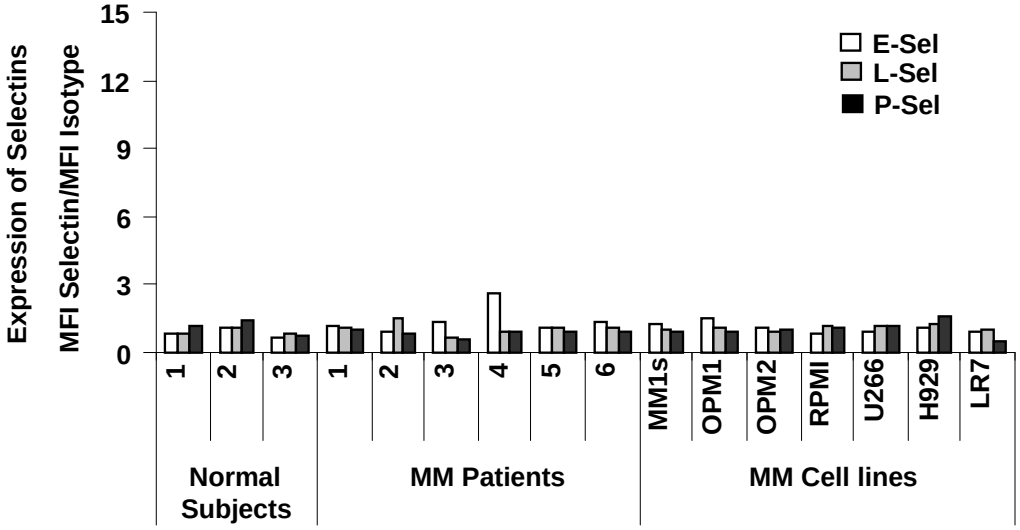

Figure S2

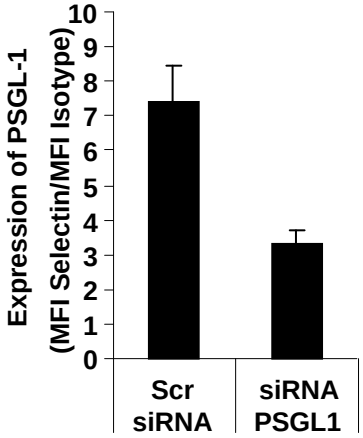

Figure S3

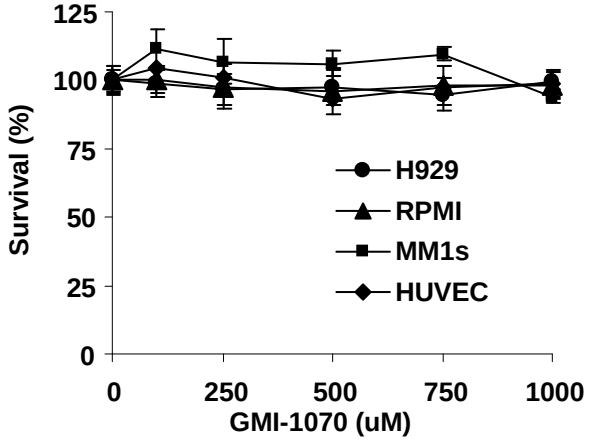

Figure S4

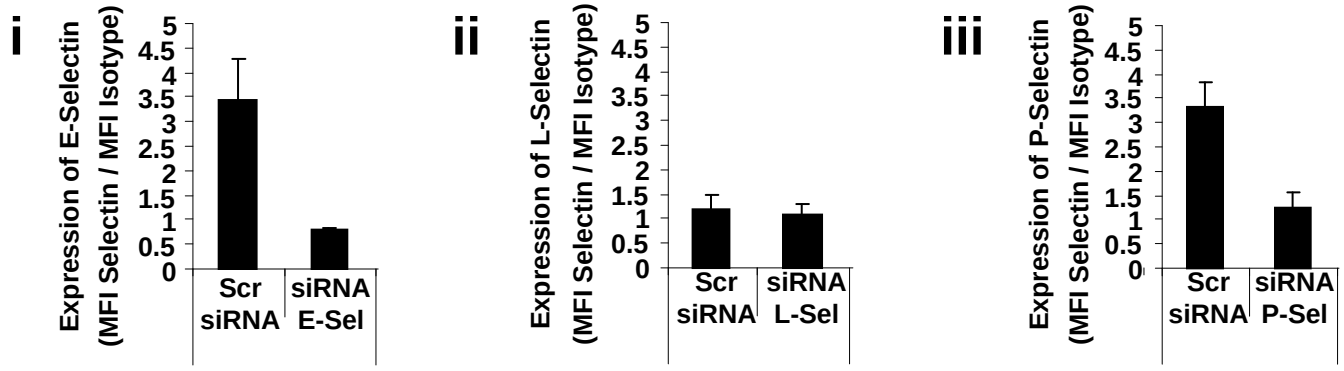

Figure S5