SUPPLEMENTARY MATERIALS AND METHODS.

Cell lines and culture conditions. Myeloma cell lines U266, RPMI8226, MM1.S were cultured in RPMI 1640 media supplemented with 10% FCS (Hyclone), antibiotic/antimycotic solution (Mediatech), MEM amino acids (Mediatech) and β-mercaptoethanol (50 μM final).

Patient sample collection and processing. Bone marrow aspirates were collected with informed consent under IRB-approved protocol (HO07403). Bone marrow aspirates from normal donors were obtained commercially from All Cells, Inc. Bone marrow aspirates were diluted with PBS and then mononuclear cells were separated using Ficoll-Hypaque 1.073 (GE Healthcare Bio-sciences, Piscataway, NJ, USA) and Leucosep tube (Greiner Bio-one, Monroe, NC, USA) according to manufacturer's protocol. RBC was lysed with 3 minute incubation in ACK lysis buffer and mononuclear cells were incubated with anti-CD138 or anti-CD14 microbeads (Miltenyi Biotec, Auburn, CA, USA) for 15 minutes. Labeled cells were isolated by using autoMACS pro cell separation system (Miltenyi Biotec, Auburn, CA, USA) and then frozen until ready for use. For mesenchymal stromal cells, the CD138/CD14- negative fraction was plated in aMEM supplemented with 10% FBS (Hyclone, Logan, UT, USA), 1X NEAA (Lonza, Walkersville, MD, USA), 2mM L-Alanine-L-Glutamine (Mediatech, Manassas, VA, USA). Attached cells were harvested and passaged using TrypLE (Invitrogen, Carlsbad, CA, USA) until reaching passage 4.

Immunoblot analysis. Whole cell lysates were prepared by boiling cells in Laemmli Sample buffer (Bio-Rad) supplemented with 100mM DTT for 10 minutes at a final concentration of 10^7 cells/ml. Alternatively cells were lysed in RIPA buffer [50mM Tris-HCL, 150mM NaCl, 1mM EDTA, 1% Triton-X, 0.5% Deoxycholate] and sonicated. Protein was quantified using Bradford assay reagent (BioRad) and then boiled for 5 minutes in Laemmli Sample buffer with 100mM DTT prior to loading. Lysis buffers were supplemented with HALT protease and phosphatase inhibitor cocktail (Thermo). 10^5 cells or 20μg protein was resolved by SDS-PAGE and transferred to Immobilon-P PVDF membrane (Millipore) or nitrocellulose membranes. Membranes were blocked in 5% Milk in TBS-T [25mM Tris-HCl (pH 7.4), 0.13M NaCl, 2.7mM KCl]. Primary antibodies were diluted in 5% Milk-TBST and membranes were incubated overnight at 4°C. Anti-phosphoprotein antibodies were typically incubated in a blocking solution of 5% BSA (Fraction V, Sigma).

The following primary antibodies were used at dilutions recommended by the manufacturers:

Anti-phospho-Thr290 TPL2 (Abcam ab51214 and Invitrogen 441370G)

Anti-total TPL2 (Santa Cruz Biotechnology sc-720)

Anti-phospho-MEK1/2 (Cell Signaling 2338)

Anti-total MEK1/2 (Cell Signaling 9126)

Anti-phospho ERK (Cell Signaling 4370)

Anti-total ERK (Cell Signaling 9102 and Cell Signaling 4696)

Anti-phospho AKT (pSer473) (Cell Signaling 4060)

Anti-total AKT (Cell Signaling 2920)

Anti-phospho p38MAPK (Cell Signaling 4631)

Anti-total p38MAPK (Cell Signaling 9212)

Anti-phosphoTACE (phospho-Thr 735) (Assay Biotech A0763)

Anti-total TACE (Cell Signaling 3976)

Anti-total phospho-histone H3 (Ser10) (Cell Signaling 9701)

Anti-GAPDH-HRP conjugate (Genscript A00192)

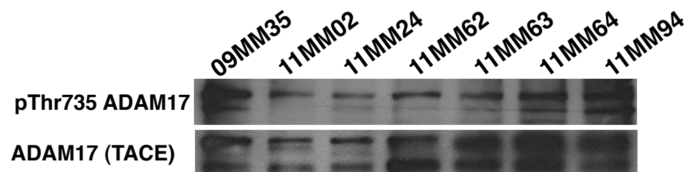
Secondary antibody [mouse anti-rabbit-HRP light-chain-specific 1:10,000 (Jackson Immunoresearch), horse anti-mouse-HRP 1:5000 (Cell Signaling)] incubations were carried out for 1 hour at room temperature. Signal detection was achieved using Amersham ECL Plus chemiluminescent solution (GE Healthcare). Blots were developed on Classic Blue Autoradiography Flim BX (MidSci).

Annexin V/ PI staining. For apoptosis detection, cells were harvested and subjected to the Annexin V staining protocol by BD Bioscience. Following staining, flow cytometry was performed within 1 hour on a FACSCalibur analyzer. For cell cycle analysis, cells were washed in PBS, fixed and permeabilized in 70% ethanol and stained with propidium iodide per standard protocols. Flow cytometry was carried out on FACSCalibur analyzer (Becton Dickinson). FlowJo software was used for flow data analysis.

SUPPLEMENTARY TABLE (CLINICAL DATA ON PATIENTS INCLUDED).

ID	Mo/Yr of sample	Year of diagnosis	Isotype	% plasma cells in BM	Prior treatments	BM cytogenetics (cyto)/ FISH
09MM35	8/2009	2007	IgAλ	10% nodules	Thal/Dex Auto	**Cyto:** Normal (20/20) **FISH:** del17p
11MM02	1/2011	2009	IgGκ	75%	Rev/Dex; Thal/Dex Double auto	**Cyto:** Hypodiploidy/ 13q monosomy (11/20) **FISH:** 13q monosomy/ 14q32 rearrangement
11MM24	3/2011	1999	IgGκ	46%	Thal; Bev/Rev/Dex; Bort/Rev/Dex Auto 2005	**Cyto:** Normal (20/20) **FISH:** del(13q)
11MM62	8/2011	2011	IgGκ	12%	None (diagnosis)	**Cyto:** Normal (20/20) **FISH:** None
11MM63	8/ 2011	2010	IgGκ	37%	None (smoldering)	**Cyto:** Normal (20/20) **FISH:** del 13q/ t(4;14)
11MM64	8/2011	2008	IgAλ	31%	Mel/Pred/Rev Bort/Dex/Doxo	**Cyto:** Normal (20/20) **FISH:** Gain 11q/ del13q
11MM94	12/2011	2001	IgGλ	55%	Bort/Dex Cytoxan Auto	**Cyto:** Complex (18/20) **FISH:** t(4;14)

Abbreviations:
Thal: Thalidomide
Dex: Dexamethasone
Rev: Lenalidomide
Bort: Bortezomib
Dox: Doxorubicin
Bev: Bevacizumab
Cytoxan: Cyclophosphamide
Auto: Autologous transplantation



Supplementary Figure.

TACE activation in myeloma-associated monocytes/macrophages.

The activation status of TACE was probed using antibodies against phosphorylated and total TACE in CD14+ mononuclear cells from myeloma bone marrows.