Primer sequences for amplification of fragments used for MITF 3′UTR luciferase reporters
UTR forward primer: 5′-TTA GCG AGC CTG CCT-3′
UTR reverse 940: 5′-TGC CCA GTT CAG TTG TCT TTA TC-3′
UTR reverse 1580: 5′-AGG CAG CCT GGC TTG TAC CA-3′
UTR reverse 2000: 5′-ATA GCA TCA TCA GTG TTT CC-3′

Real time PCR for MITF
Primers for MITF and beta actin used at a concentration of 200 nM and PCR performed at 94° for 30 seconds, 55° for 30 seconds and 72° for 30 seconds for 35 cycles. Primer sequences are as follows: MITF forward 5′-GTG CAG ACC CAC CTG GAA AAC-3′ MITF reverse 5′-AGT TAA GAG TGA GCA TAG CCA TAG-3′. Beta actin forward 5′-CAG CCA TGT ACG TAG CCA TCC-3′, beta actin reverse 5′-TTT GAT GTC ACG CAC GAT TTC C-3′. Expression was normalized to Beta actin as an internal control so that $\Delta\Delta Ct = (Ct_{MITF\ treated} - Ct_{beta\ actin}) - (Ct_{MITF\ no\ treatment} - Ct_{beta\ actin})$. MITF treated refers to samples treated with SCF or imatinib. MITF no treatment refers to the samples treated with SCF at the zero time point or no imatinib.

PCR for c-Kit mutations
c-DNA was reverse-transcribed from total RNA from mononuclear cells obtained from patient bone marrow samples. A 373-bp region of the c-KIT gene was amplified by PCR using the following primers: primer1 (kitAsp816F 5′-AAA GGA GAT CTG TGA GAA TAG GCT C-3′) and primer2 (kitAsp816R: 5′-AGC TCC CAA AGA AAA ATC CCA TAG G-3′). PCR products were digested with the restriction enzymes Hae III and Hinf I (New England Biolabs, Ipswich, Mass) and electrophoresed on a polyacrylamide gel (Invitrogen)

Primers sequences for amplification of miR cluster:
Forward human miR cluster: 5′-GGA ACA AGT TCT AAT CTG GAG TGA TGC C-3′
Reverse human miR cluster: 5′-CCT AAA TGG CAT CTC CAC CC-3′

shRNA stemloop sequences for construction of pLL3.7 constructs:
A detailed protocol describing the cloning of si RNA constructs inot pLL3.7 can be found at Dr. Tyler Jack's website: http://web.mit.edu/jacks-lab/protocols/pll37.htm. The algorithm used for the design of stem loop sequences is described by Dr. Tom Tuschl at http://www.mpibpc.gwdg.de/abteilungen/100/105/sirna.html. The sequences are shown below. The stem loop sequences are underlined and the hairpin loop is in lowercase letters. The MITF stem loop sequences is complimentary to both the human and mouse MITF sequence.

si MITF sense: 5′-TGC AGT ACC TTT CTA CCA CTt tca aga GAA GTG GTA GAA AGG TAC TGC TTT TTT C-3′

si MITF antisense: 5′-TCG AGA AAA AAG CAG TAC CTT TCT ACC ACT tct ctt gaa AGT GGT AGA AAG GTA CTG CA-3′

si β-actin sense: 5′-TTG AAG ATC AAG ATC ATT GCt tca aga GAG CAA TGA TCT TGA TCT TCA TTT TTT C-3′

si β-actin antisense: 5′-TCG AGA AAA AAT GAA GAT CAA GAT CAT TGC tct ctt gaa GCA ATG ATC TTG ATA TTC AA-3′

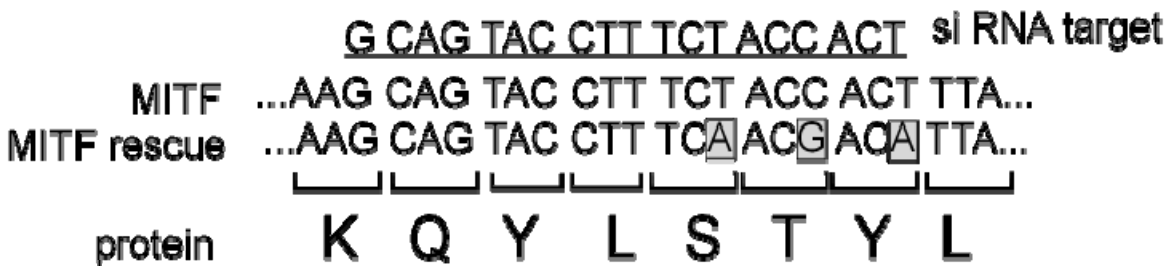


Figure S1. MITF expression cDNA resistant to siRNA silencing
The MITF sequence targeted by the sh-RNA expressing lentivirus is underlined. Three nucleotide changes (grey boxes) were introduced into wobble positions of the MITF cDNA resulting in cDNA that expresses wild type MITF protein, but is resistant to si RNA silencing (MITF rescue).

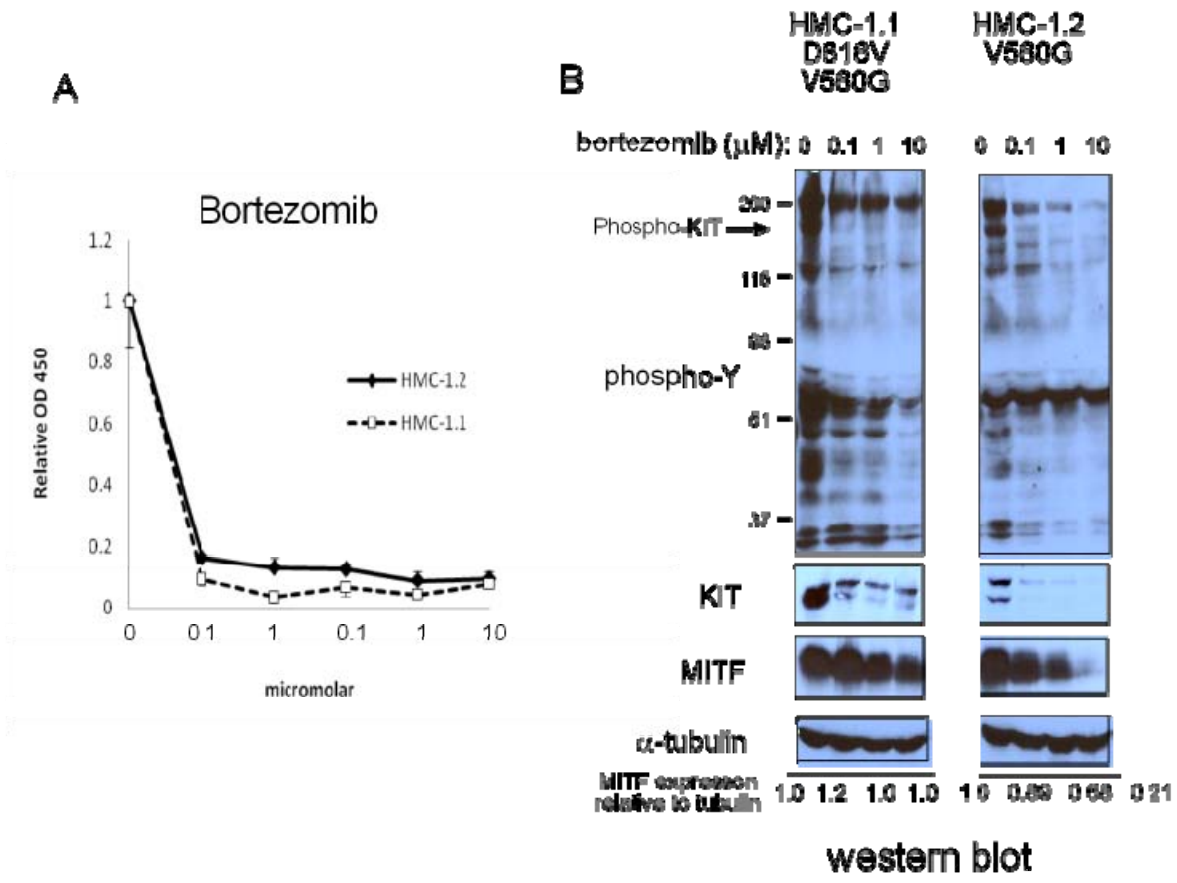


Figure S2. Differential effects of proteosome inhibitor bortezomib on MITF expression in neoplastic mast cells

(A) D816V negative (HMC-1.1) and positive (HMC-1.2) cell lines are sensitive to bortezomib. XTT assays were performed in triplicate and absorbance measured at OD 450. Relative OD 450 value is calculated by normalizing OD 450 reading to OD450 with no treatment. Experiments performed in triplicate. (B) MITF protein expression is significantly reduced in D816V positive cells treated with bortezomib, but not in D816V negative cells. Western blot shows that both phosphotyrosine signal and KIT protein is reduced with bortezomib treatment in HMC-1.1 and HMC-1.2, but MITF protein is significantly reduced only in HMC-1.2. Western blot for α-tubulin shows equivalent loading. Relative expression is calculated by taking the ratio of the densitometry signal for MITF to tubulin and normalizing to 1.0 for the zero time point.

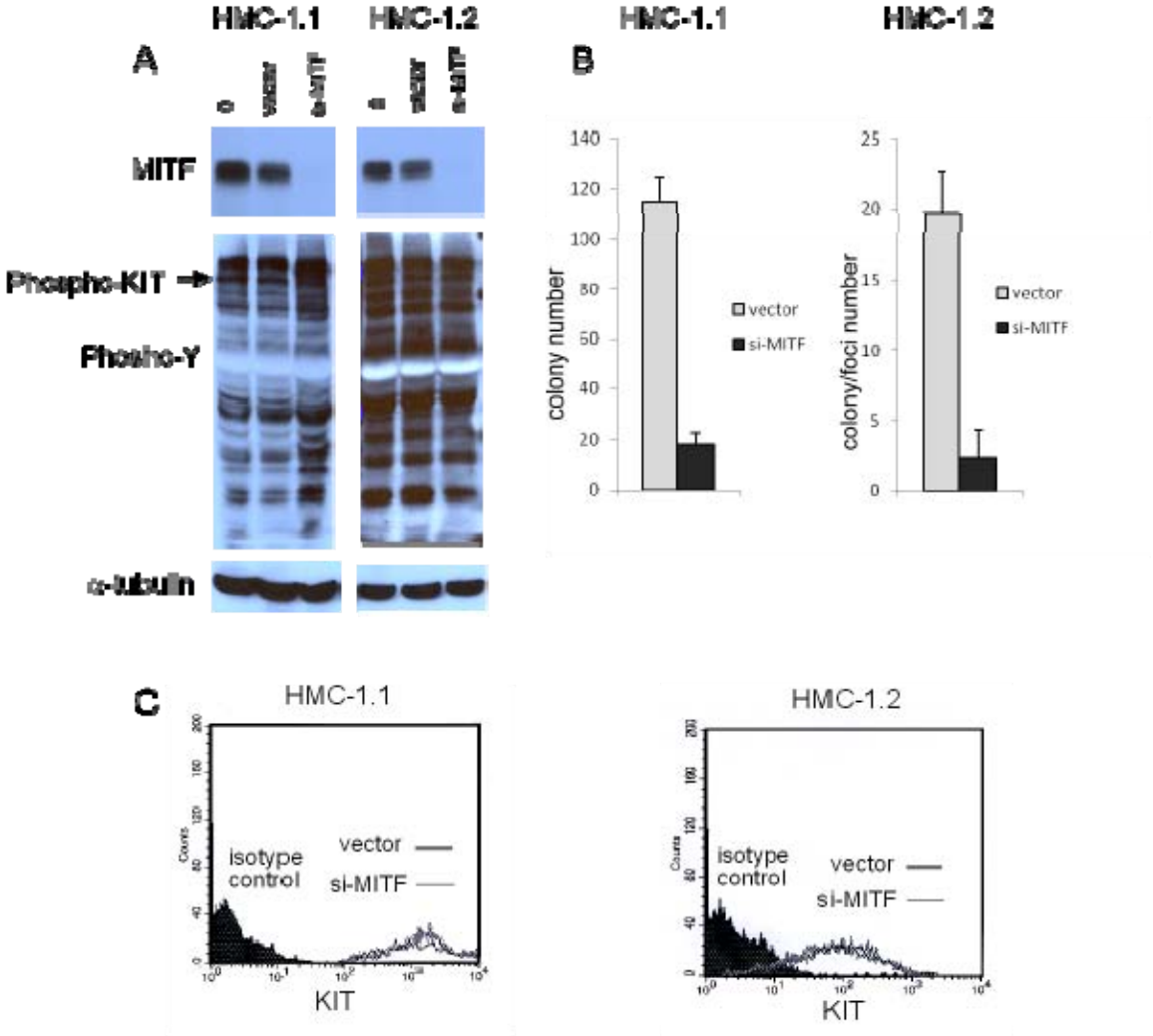


Figure S3. MITF knockdown inhibits cell growth in D816V positive and D816V negative mast cells, but does not affect KIT function or expression

(A) Western blot shows efficient knockdown of MITF protein expression with sh-RNA expressing lentivirus (si MITF) in D816V negative (HMC-1.1) and D816V positive (HMC-1.2) mast cell lines. No lentivirus or empty lentivirus shows no effect on MITF expression. Phosphotyrosine blot show no inhibition of KIT phosphorylation in si MITF transduced cells. (B) MITF repression significantly impairs colony forming capacity in both HMC-1.1 and HMC-1.2 mast cells lines. Cells were plated in 1% methylcellulose and GFP positive colonies were counted on day 12 for HMC-1.1 cells. HMC-1.2 cells did not form colonies as efficiently as HMC-1.1, but tended to form adherent foci. GFP positive HMC-1.2 colonies and foci were counted. Experiments were performed in triplicate. (C) MITF knockdown does not affect KIT expression in HMC cells. HMC cells were stained with PE-labeled antibodies to human KIT. PE-labeled isotype control antibodies were used for negative control. GFP positive cells were gated, and KIT signal was measured. Cells with si-MITF lentivirus showed no difference in KIT expression compared to vector treated cells.

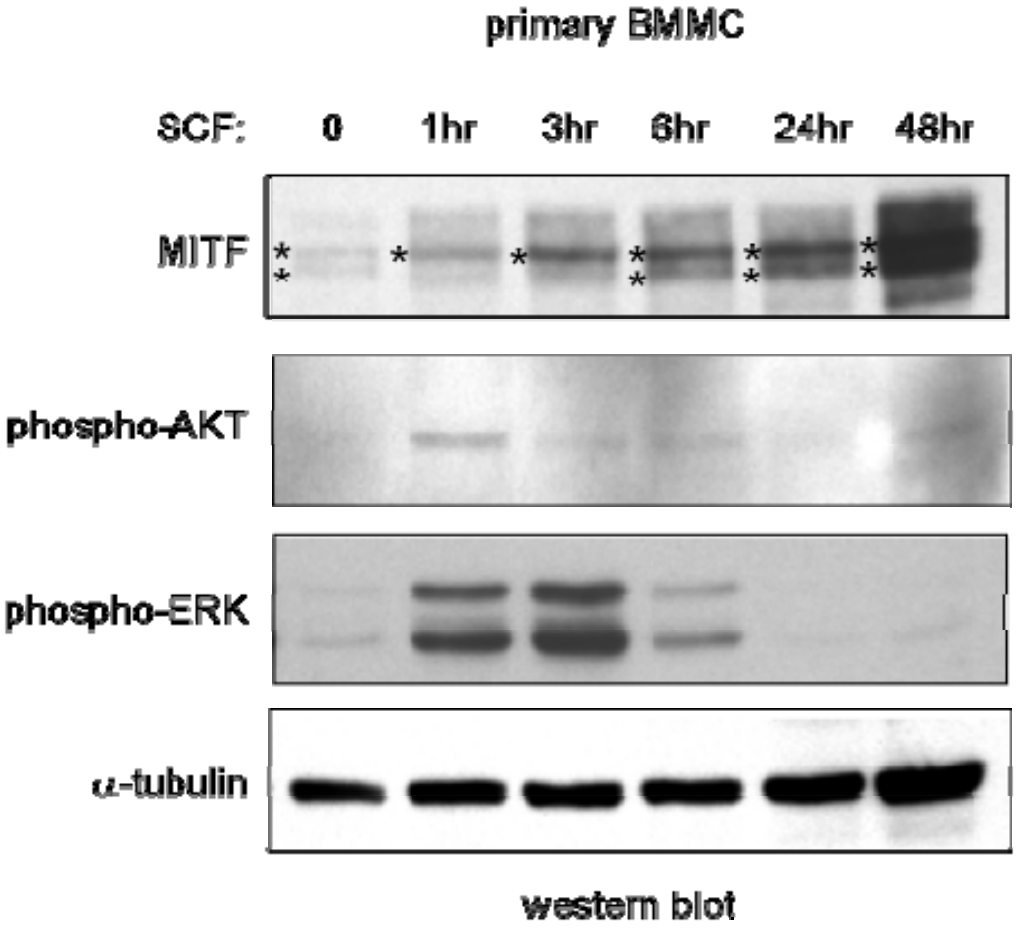
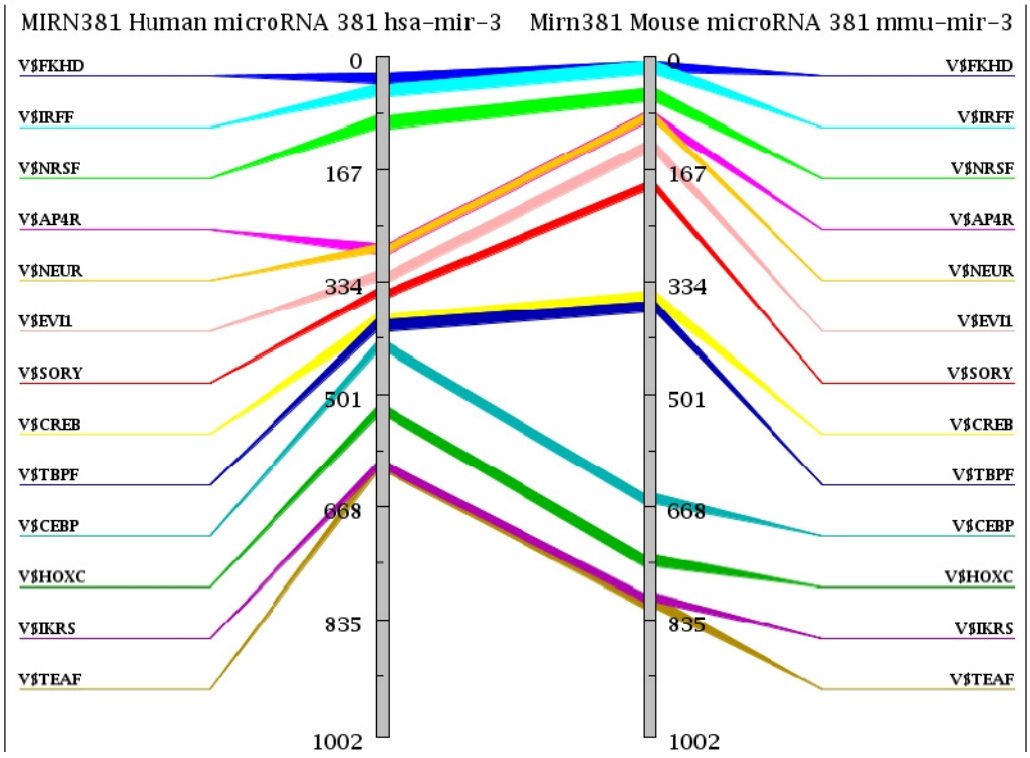


Figure S4. SCF treatment results in transient mobility shift of MITF protein, as well as increased protein levels

BMMCS were starved of cytokine, and treated with 100 ng/cc of SCF for various time points. Multiple isoforms and phosphorylation states result in several MITF bands on western blot. At baseline, 2 prominent bands are noted (denoted by *). At 1 and 3 hours, the upper band is prominent; by 24 hours the band pattern returns to baseline. Total MITF protein is also markedly increased by 24 to 48 hours. SCF treatment also results in rapid and transient phosphorylation of AKT and ERK as shown by phopho-AKT and phosphor-ERK blots. α-tubulin is shows loading of protein.

A

Genome Trafac Identification of ConservedTranscription Factor Binding Sites
1000 base pairs upstream miR-381



V$FKHD: Xenopus forkhead domain factor 3 (FoxA2n)

V$IRFF: Interferonstimulated response element

V$NRSF: Neuralrestrictivesilencer-element

V$AP4R: Tal-1alpha E47 heterodimer

V$NEUR: Neurogenin 1 and 3 (ngn1 3) binding sites

V$EVI1: Ecotropic viral integration site 1 encoded factor

V$SORY: HMG boxcontaining protein 1

V$CREB: E-IBP4. bZIP domain. transcriptional repressor

V$TBPF: Mammalian Ctype LTR TATA box

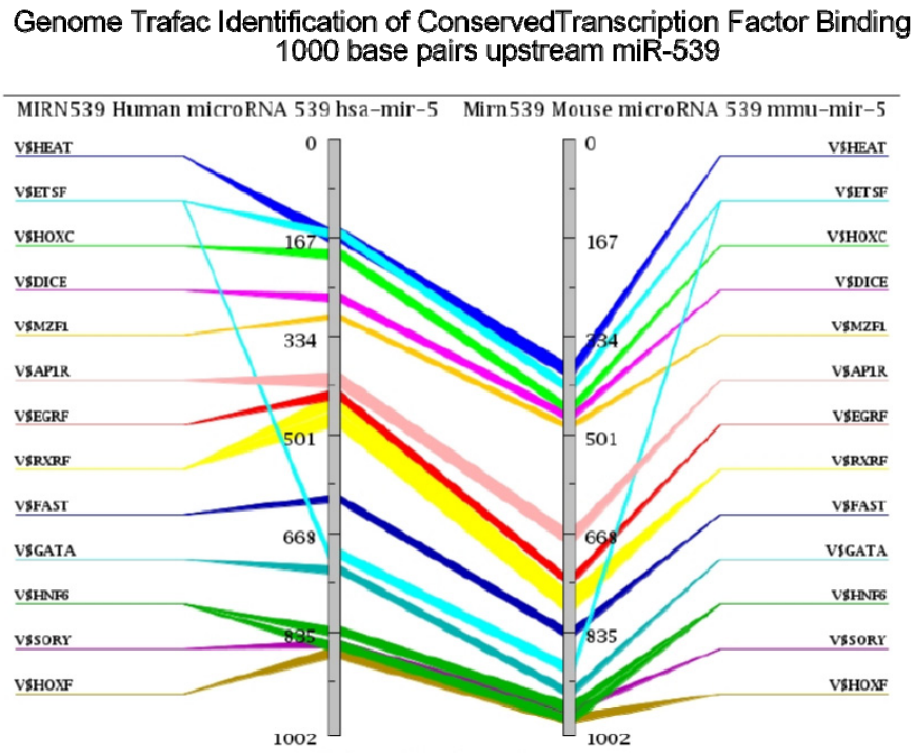
V$CEBP: CCAAT enhancer binding protein beta

V$HOXC: Homeo domain factor Plx-1

V$IKRS: Ikaros 1. potential regulator of lymphocyte differentiation

V$TEAF: TEF-1 related muscle factor

B



V$HEAT: Heat shock factor 1

V$ETSF: Prostate-derived Ets factor

V$HOXC: HOX PBX binding sites

V$DICE: Downstream Immunoglobulin Control Element

V$MZF1: Myeloid zinc finger protein MZF1

V$AP1R: binding to subclass of AP1sites

V$EGRF: Collagen krox protein (zinc finger protein 67 - zfp67)

V$RXRF: Bipartite binding site of VDR RXR heterodimers

V$RXRF: Bipartite binding site of VDR RXR heterodimers

V$FAST: FAST-1 SMAD Interacting protein

V$ETSF: c-Ets-1 binding site

V$GATA: Complex of Lmo2 bound to Tal-1, E2A proteins, and GATA-1

V$HNF6: Liver enriched Cut –Homeodomain transcription factor HNF6

V$SORY: Sox-5

V$HNF6: Liver enriched Cut –Homeodomain transcription factor HNF6

V$HOXF: Phox2a (ARIX) and Phox2b 857

Figure S5. Computational analysis identifies conserved, putative transcription factor binding sites in upstream genomic regions for miR-381 and miR-539

(A) Analysis of 1KB genomic region upstream of miR-381 using Genometrafac (http://genometrafac.cchmc.org/) identifies putative transcription factor binding sites conserved between mouse and human. Transcription factor names for abbreviations listed below. (B) Similar analysis of 1KB genomic region upstream of miR-539 with transcription factor names listed below.