Supplementary Methods

Probes and oligonucleotides used for quantitative RT-PCR

TaqMan probes (Applied Biosystems) used to detect mRNA levels: *GAPDH* (Hs99999905_m1), *PEDF* (Hs00171467_m1), *TYR* (Hs00165976_m1) and *MITF* (Hs00165156_m1).

Oligonucleotides used to detect hnRNA levels: *PEDF* (sense 5'-CCCTCGAGGATTTCTACT TGG-3' and antisense 5'-GCTGTCGGATCTCAAAGGTC-3'), *MITF* (sense 5'-CGAAAGTTGCAACGAGAACA-3' and antisense 5'-GTCAACTCCCCTATGGCTCA-3') and *TYR* (sense 5'-GAGTACATGGGAGGTCAGCA-3' and antisense 5'-AACAGGGCACCATTTCTGTC-3').

Over-expressing lentivirus vector

For MITF over-expression, we generated a lentiviral construct encoding the full-length human MITF cDNA inserted between EcoRI and NotI sites of the pCDH-CMV-MCS-EF1-copGFP (pCDH) lentiviral vector. MITF cDNA was amplified by PCR from pCDNA3.1-HA-MITF plasmid (provided by D. Fisher, Cutaneous Biology Research Center, Charlestown, MA, USA); EcoRI and NotI sites were added to 5' and 3' ends, respectively, using primers 5-CGGGAATTCATGGCCTACCCATACGACG-3' and 5'-CGGGCGGCCGCCCTAACAAGT GTGCTCC-3'. This fragment was then subcloned into TA cloning vector (Invitrogen, Carlsbad, CA, USA), digested with EcoRI and NotI and re-cloned in the pCDH plasmid digested with EcoRI and NotI.

For OIS induction by $HRAS^{G12V}$ over-expression in melanocytes, we generated a lentiviral construct encoding $HRAS^{G12V}$ cDNA inserted between XbaI and XhoI sites of the prrl.CMV.EGFP.wpre.SIN (pRRL) lentiviral vector. $HRAS^{G12V}$ cDNA was amplified by PCR from FG12-eGFP-$HRAS^{G12V}$ (provided by M. Soengas, CNIO, Madrid, Spain). XbaI and XhoI sites were added to 5' and 3' ends, using primers 5'-CCGTCTAGATACCCATACGATGTTCCTGA-3' and 5'-CCGTCGAGTGAGGAGAGCCAC ACACTTGC-3'. This fragment was subcloned into TA cloning vector (Invitrogen), digested with XbaI and XhoI, and re-cloned in the pRRL plasmid digested with XbaI and XhoI.

Plasmid construction

Human genomic DNA extracted from human blood cells was used as template for PCR amplification of three MITF binding sites to PEDF. BamH1 sites were added to 5’ and 3’ ends using the following primers (sites named in order to their proximity to ATG origin): site A 5’CGGGGATCCGTCTCAGCAGGATTCCCAGA-3’ and 5’-CGGGGATCCCCTCAGCACA CACGCAATACC-3’; site B 5’-CGGGGATCCTGCCTCCTGTGTACCTCACT-3’ and 5’-CGGGGATCCTGATTGTTGGCTCTCGTCCTTC-3’; site C 5’-CGGGGATCCCCACTGTGC TCGGTAGTTGTT-3’ and 5’-CGGGGATCCGGTCAGAGATCCTGGGTTCA-3’. PCR products were cloned into BamH1 sites of prolactin- vector [1].

Supplementary references

[1] Rincon M, Flavell RA (1994). AP-1 transcriptional activity requires both T-cell receptor-mediated and co-stimulatory signals in primary T lymphocytes *EMBO J* **13**, 4370-4381.

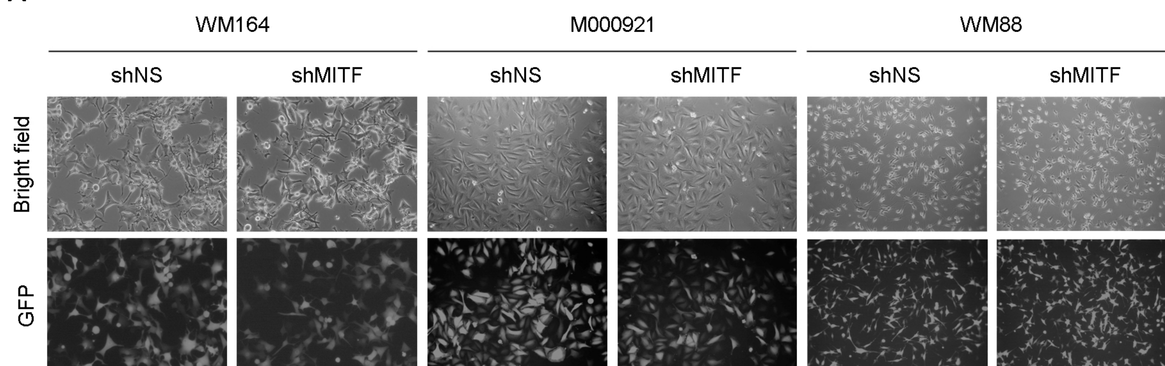
Supplementary Table 1

ANTIGEN	ORIGIN	DILUTION WB	DILUTION IF	SOURCE
PEDF	Rabbit polyclonal	1:1500	1:100	Bioproducts, West Palm Beach, FL, USA
MITF	Mouse monoclonal, clone D5	1:200		Sigma, St Louis, MO, USA
MITF	Mouse monoclonal, clone D5	1:200	1:50	Thermo Scientific, Fremont, CA, USA
RAS	Mouse monoclonal	1:2000		Calbiochem, San Diego, CA, USA
BRAF	Mouse monoclonal	1:500		Santa Cruz Biotechnologies, Santa Cruz, CA, USA
HA	Mouse monoclonal	1:500		Roche, Konzern Hauptsitz, Switzerland
$p16^{INK4A}$	Mouse monoclonal	1:500		Santa Cruz Biotechnologies, Santa Cruz, CA, USA
H3K9Me	Rabbit polyclonal		1:2000	Upstate Cell Signaling Solutions, Lake Placid, NY, USA
β-tubulin	Mouse monoclonal	1:10000		Sigma, St Louis, MO, USA
β-actin	Goat polyclonal	1:1000		Santa Cruz Biotechnologies, Santa Cruz, CA, USA

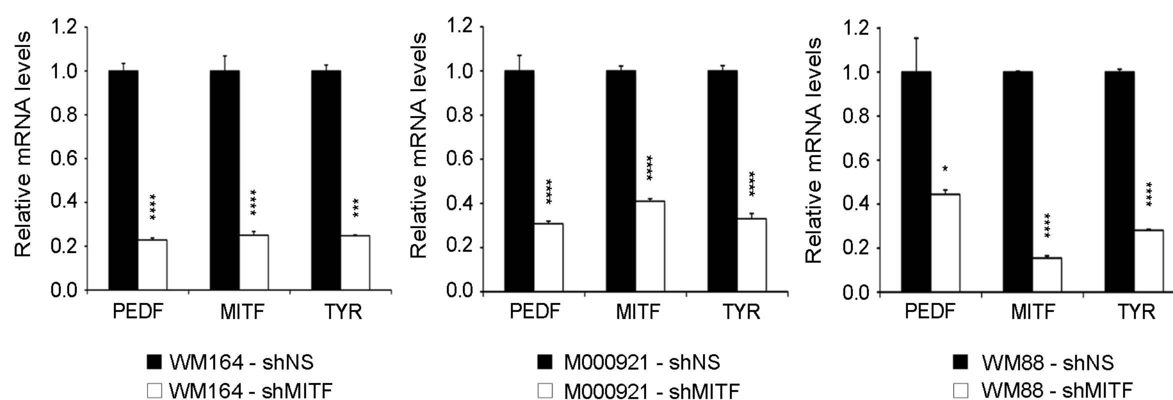
Abbreviations used: WB, Western blot; IF: Immunofluoresecence; PEDF, pigment epithelium-derived factor; MITF, microphthalmia-associated transcription factor

Supplementary Figure 1.

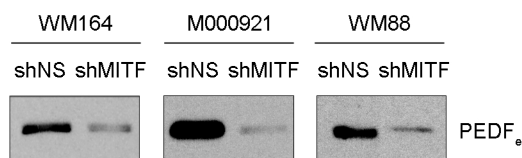
A



B

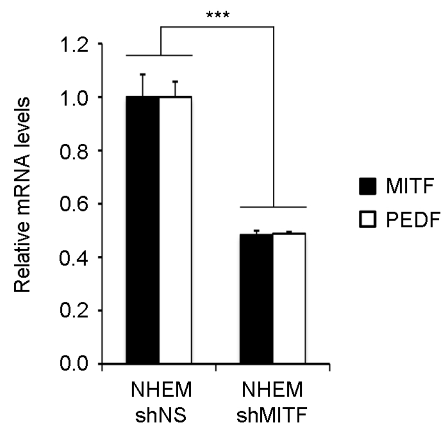


C



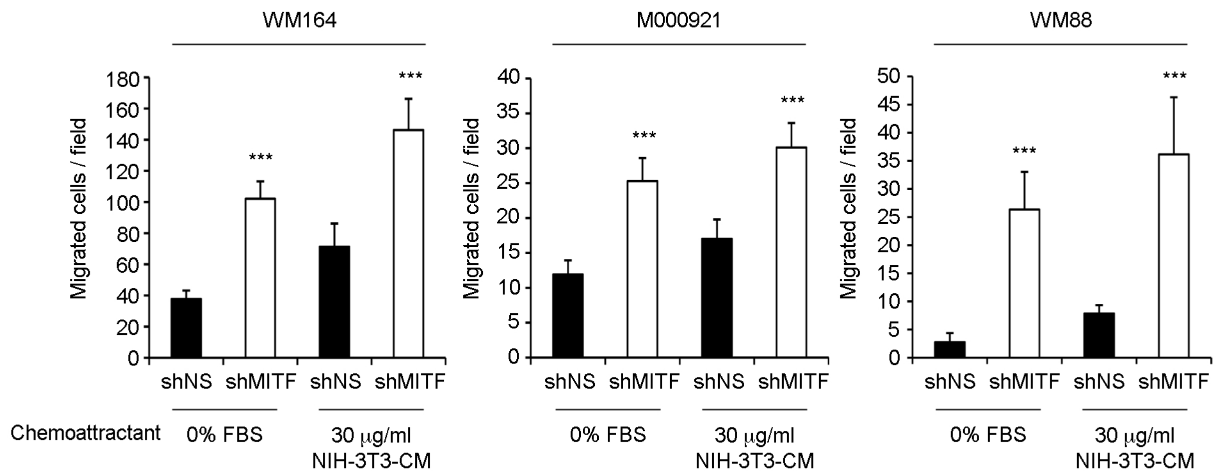
Supplementary Figure 1. MITF interference in weakly aggressive melanoma cell lines down-regulates PEDF expression. (A) Transduction efficiency of WM164, M000921 and WM88 melanoma cell lines after infection with non-silencing (shNS) or shRNA^{mir} to MITF (shMITF) lentivirus at multiplicity of infection (MOI) of 80 (WM164), 10 (M000921) or 20 (WM88). Fluorescence images (10x magnification) show more than 90% GFP-positive cells. (B) Quantitative RT-PCR of PEDF, MITF and TYR mRNA levels in WM164 (left), M000921 (middle) and WM88 (right) shNS and shMITF melanoma cell lines. PEDF, MITF and TYR mRNA levels are shown relative to control shNS cells after normalization to GAPDH mRNA levels. Bars represent average +/- standard deviation (SD), and statistical significance was determined by Student's test (*P<0.05; ***P<0.001; ****P<0.0001). (C) Western blot analysis of extracellular PEDF (PEDF$_e$) protein levels in concentrated conditioned medium (CM) from WM164, M000921 and WM88 shNS and shMITF melanoma cell lines.

Supplementary Figure 2.



Supplementary Figure 2. MITF interference down-regulates PEDF expression in melanocytes. Quantitative RT-PCR of PEDF and MITF mRNA levels in primary melanocytes (NHEM) transduced with non-silencing (shNS) or shRNA^{mir} to MITF (shMITF) lentivirus at multiplicity of infection (MOI) of 40. PEDF and MITF mRNA levels are shown relative to control shNS cells after normalization to GAPDH mRNA levels. Bars represent average +/- standard deviation (SD). Statistical significance was determined by Student's test (***P<0.001).

Supplementary Figure 3.



Supplementary Figure 3. MITF interference increases migration of weakly aggressive melanoma cell lines. Migration assays of WM164 (left), M000921 (middle) and WM88 (right) shNS and shMITF melanoma cell lines toward 0% FBS and 30 µg/ml of concentrated conditioned medium (CM) from NIH-3T3 cells for 16 h (WM164) or 24 h (M000921 and WM88). Statistical significance was determined by one-way ANOVA using Tukey-Kramer post-test (***P<0.001).