

Figure W1. Silencing of gene targets. Quantitative RT-PCR of total mRNA samples isolated from A375 cells transduced with lentiviral vectors encoding shRNA targeting DOCK3, WAVE2, ARHGAP22, LR, PEDF-R, or nonsilencing control (NS).

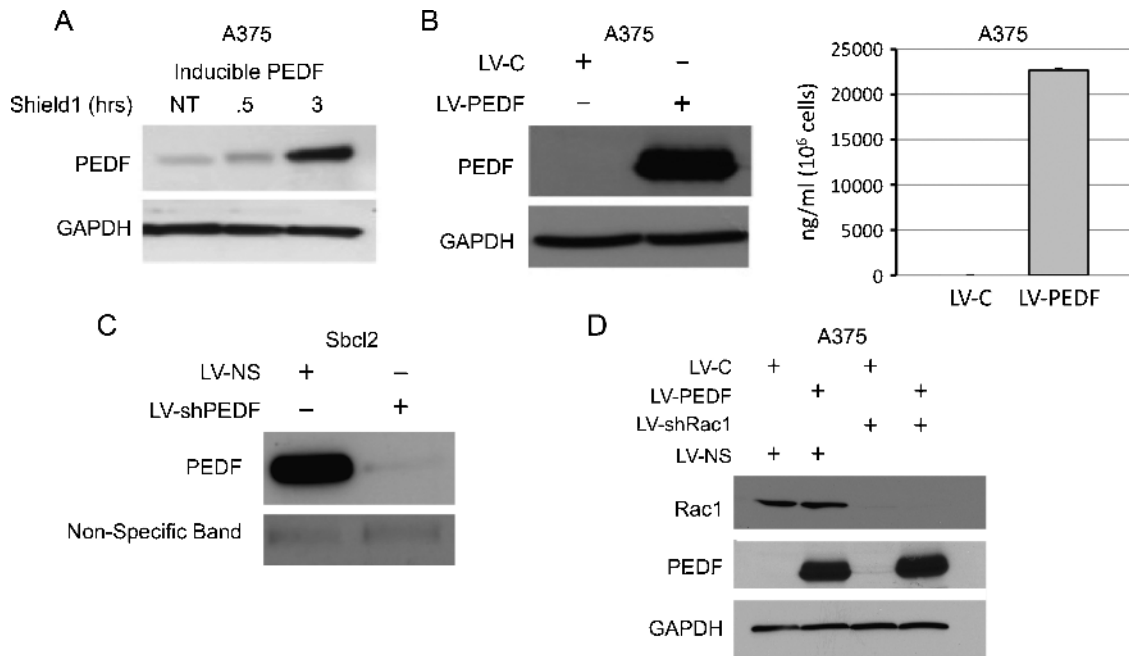


Figure W2. Manipulation of PEDF and Rac1 expression. (A) Western blot measuring PEDF expression A375 lysate transduced with inducible PEDF (iPEDF) and treated with 250 nM shield1 (inducer) for the indicated time points. (B) Western blot probing for PEDF in cell lysate and ELISA data (right) quantifying PEDF expression in the conditioned medium of A375 melanoma transduced with bicistronic lentivirus expressing LV-C (GFP only) or LV-PEDF (GFP and PEDF). (C) Western blot on conditioned medium of SBcl2 melanoma transduced with nonsilencer control (LV-NS) or shRNA targeting PEDF (LV-shPEDF). (D) Western blot for Rac1 in A375 LV-C and LV-PEDF lysate transduced with lentivirus encoding shRac1 (LV-shRac1) or nontargeting vector (LV-NS).

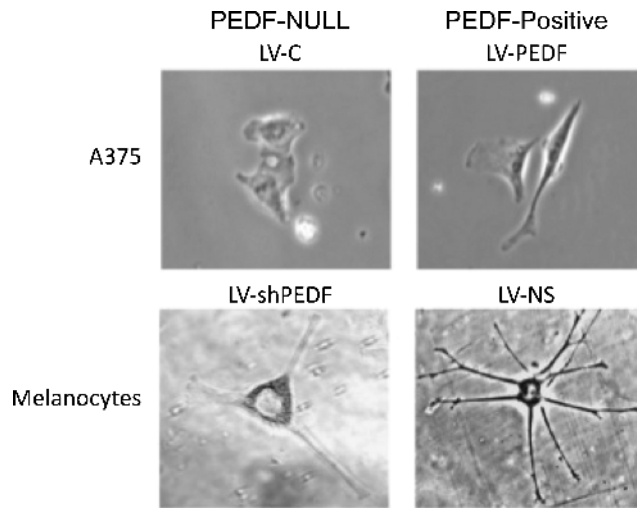


Figure W3. PEDF controls cell shape. Images of A375 melanoma (top) and melanocytes (bottom) transduced with LV-C or LV-PEDF (A375) and LV-NS or LV-shPEDF (melanocytes).

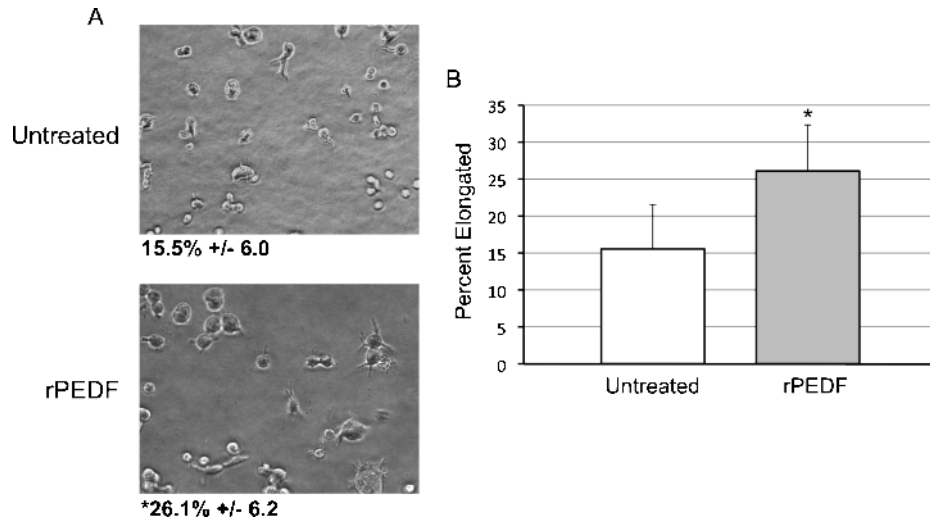


Figure W4. Exogenous PEDF promotes an elongated morphology. Amoeboid/mesenchymal morphology assay measuring the ability of 10 nM recombinant PEDF (rPEDF) to promote an elongated morphology in A375 melanoma. (A) Representative image of cells grown on a thick layer of collagen and (B) quantification. * $P < .05$. Error bars, SD.

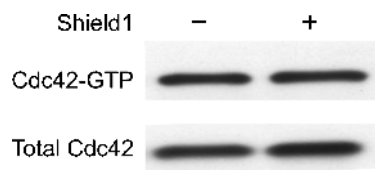


Figure W5. PEDF expression does not effect cdc42 activity. Pull-down assay measuring cdc42 activity in A375 iPEDF 30 minutes after PEDF induction with shield1.

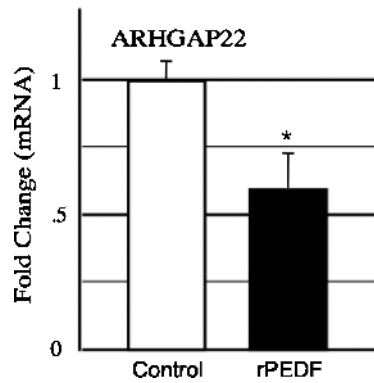


Figure W6. PEDF represses ARHGAP22. QRT-PCR of cDNA from A375 treated overnight with 10 nM recombinant PEDF compared with control vehicle.

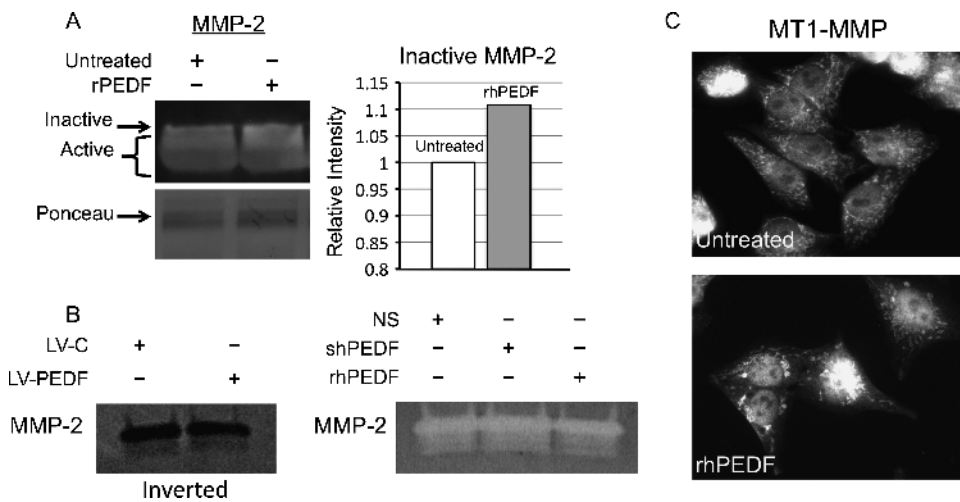


Figure W7. Exogenous PEDF redistributes MT1-MMP and moderately suppresses MMP-2. (A) Western blot (left) on the conditioned medium of A375 melanoma treated with 10 nM PEDF and a quantification (right) of the inactive MMP-2 band. No difference was seen in total MMP-2. (B) Gelatin zymography of A375 and SBcl2 melanoma with the indicated treatments. (C) Immunofluorescence for MT1-MMP in A375 treated with exogenous PEDF. A concentration of 10 nM PEDF was used in all experiments.

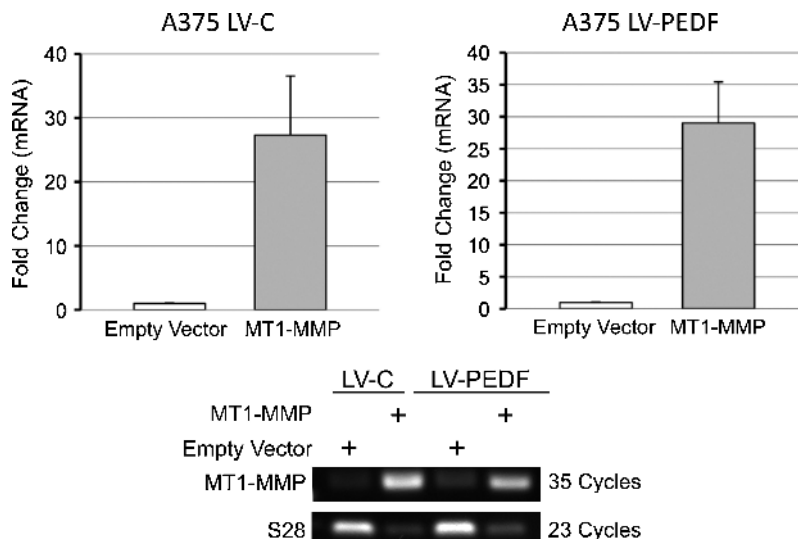


Figure W8. MT1-MMP overexpression. Measurement of MT1-MMP overexpression in A375 by qRT-PCR (top) and conventional RT-PCR (bottom) after transfection with plasmid (pcDNA3.1(+)) encoding MT1-MMP.

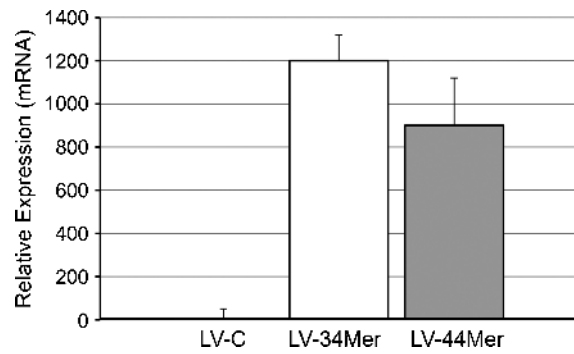


Figure W9. Relative expression of 34-mer and 44-mer. Quantitative RT-PCR measuring the relative expression of forced 34-mer and 44-mer by lentivirus. The graphs indicate the fold increase compared the LV-C.

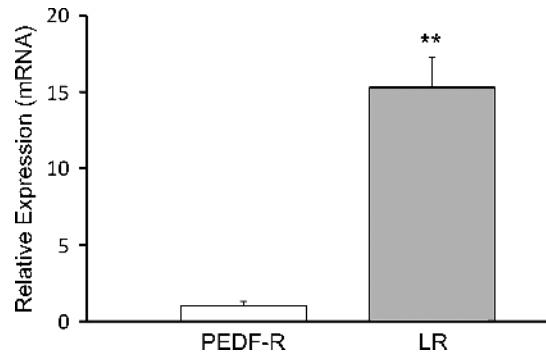


Figure W10. Relative expression of PEDF-R and LR. Quantitative RT-PCR of cDNA extracted from A375 LV-C melanoma measuring the relative expression of PEDF-R and LR.

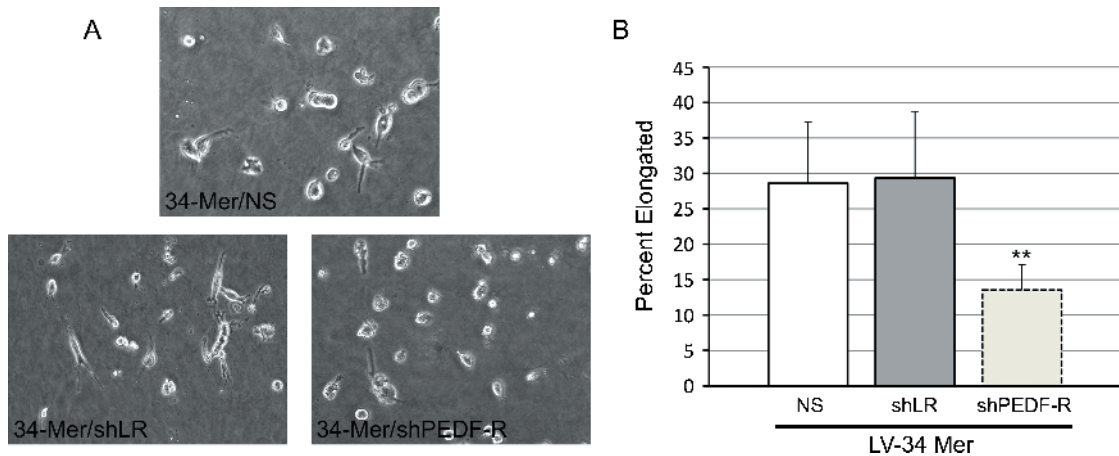


Figure W11. PEDF-R is required for 34-mer-induced elongated morphology. Amoeboid/mesenchymal morphology assay in A375 melanoma overexpressing the PEDF, 34-mer, and infected with lentivirus encoding shRNA targeting LR, PEDF-R, or nonsilencing control. Representative image (A) and quantification (B) are shown. ** $P < .01$.