Supplemental Information

Figure S1. B-RAF regulates melanoma cell shape. (A) Phase-contrast images of WM793 treated with control, B-RAF$^{\#1}$ or B-RAF^{V600E} siRNA. (B) Quantification of cell shape performed on phase-contrast images obtained using an Olympus BMX-60 inverted microscope equipped with a cooled CCD sensi-camera (Cooke) and Image-Pro Plus software (Media Cybernetics Inc., Silver Springs, MD). Cell area measurements were determined using NIH image software.

Figure S2. Activity of siRNA-resistant myc-B-RAF^{V600E}. COS-7 cells were co-transfected with pCMV5-EE-MEK1 and either pEF-myc empty vector, pEF-mycB-RAF^{V600E} or a siRNA-resistant form of pEF-mycB-RAF^{V600E} (pEF-mycB-RAF^{V600E*}). Twenty-four hours post-transfection, cells were lysed and lysates probed by western blotting with antibodies to the myc-tag, phospho-MEK1/2 and MEK1.

Figure S3. B-RAF regulates actin organization, cofilin phosphorylation and Rnd3 expression in WM115 melanoma cells. B-RAF^{V600D}-expressing, vertical growth phase WM115 cells were transfected with control siRNA or duplexes targeting total B-RAF (B-RAF$^{\#1}$ and B-RAF$^{\#2}$). The B-RAF$^{\#2}$ siRNA has been previously described (Goodall et al., 2004). (A) Cell lysates were analyzed by western blotting for B-RAF, phospho-MEK1/2, and MEK1. (B) F-actin organization was visualized using TRITC-conjugated phalloidin. Bar, 50 μm. (C) Cell lysates were analyzed by western blotting for B-RAF, Rnd3, phospho(S3)-cofilin and total cofilin.

Figure S4. Cyclin D1 knockdown does not enhance stress fiber formation in WM793 cells. WM793 cells were transfected with control or cyclin D1 siRNA. (A) Cell lysates were analyzed by western blotting for levels of cyclin D1 and tubulin. (B) F-actin organization was analyzed by TRITC-phalloidin staining. Bars, 20 μm.

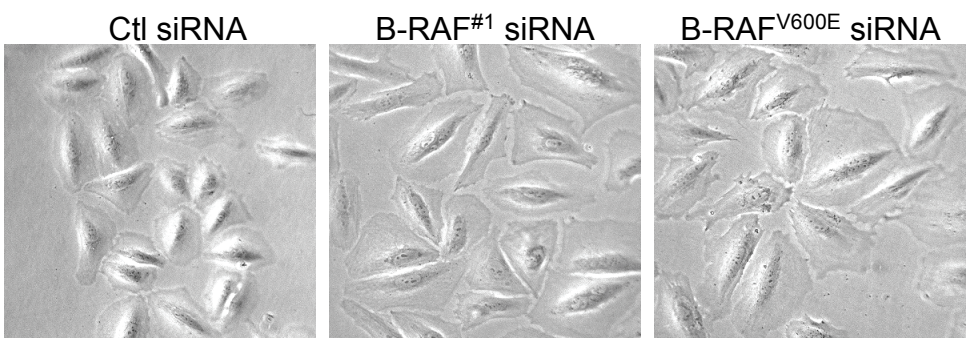
Figure S5. B-RAF knockdown stabilizes focal adhesions. WM793 cells were transfected with (A and C) control siRNA or (B and D) B-RAF$^{\#1}$ siRNA for 72 hours. Subsequently, cells were transfected to express GFP-vinculin for 24 hours prior to acquiring time-lapse images of vinculin-containing focal adhesions. The GFP-vinculin dynamics were acquired every two minutes over a 20 minute time interval. Images shown in (A) and (B) are magnified images of the whole cells shown in (C) and (D), respectively.

Figure S6. B-RAF knockdown does not influence ROCKII expression or actin organization in human foreskin fibroblast (HFF) cell cultures. HFFs treated with control or B-RAF duplexes (#1 or #2) for 72 hours. (A) Cell lysates were analyzed by western blot for B-RAF, ROCKII and MEK1 protein levels. (B) F-actin organization was visualized in HFFs by staining with TRITC-conjugated phalloidin. Bar, 50 μm.

Reference

Goodall, J., Wellbrock, C., Dexter, T. J., Roberts, K., Marais, R. and Goding, C. R. (2004). The Brn-2 transcription factor links activated B-RAF to melanoma proliferation. Mol. Cell. Biol. *24*, 2923-2931.

A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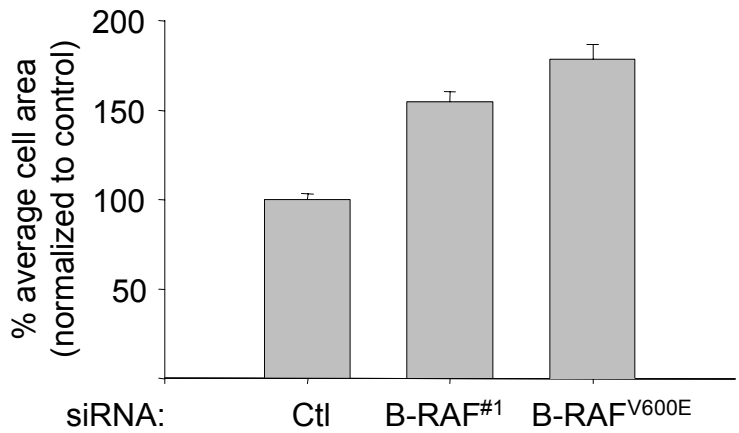
B-RAF#1 siRNA
B-RAFV600E siRNA

B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(normalized to control)
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150
100
50
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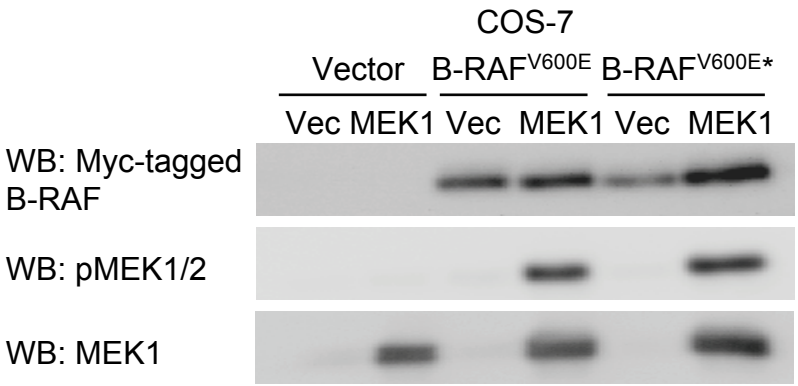
B-RAF#1
B-RAFV600E


COS-7
Vector
B-RAF^{V600E}
B-RAF^{V600E*}
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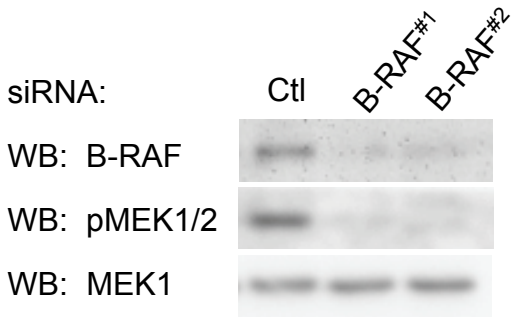
WB: Myc-tagged B-RAF
WB: pMEK1/2
WB: MEK1

A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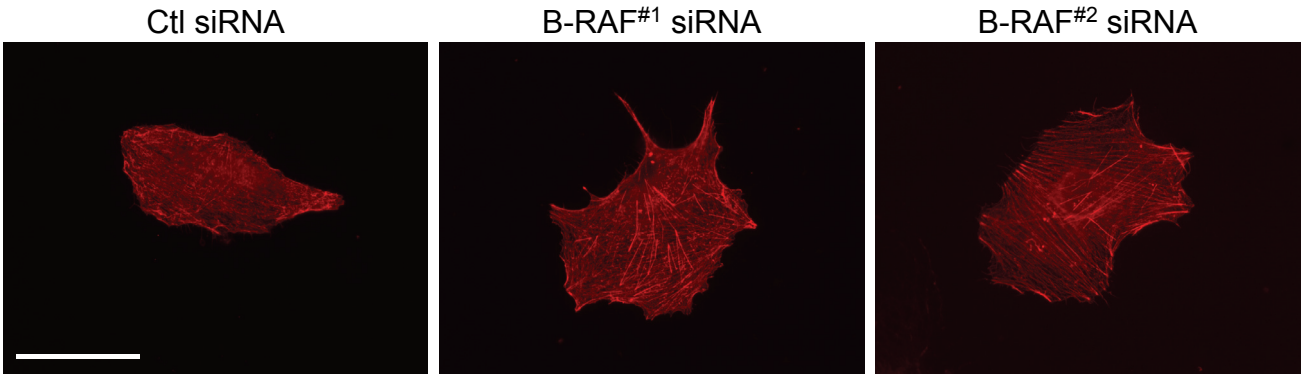
Ctl
B-RAF#1
B-RAF#2
WB: B-RAF
WB: pMEK1/2
WB: MEK1

B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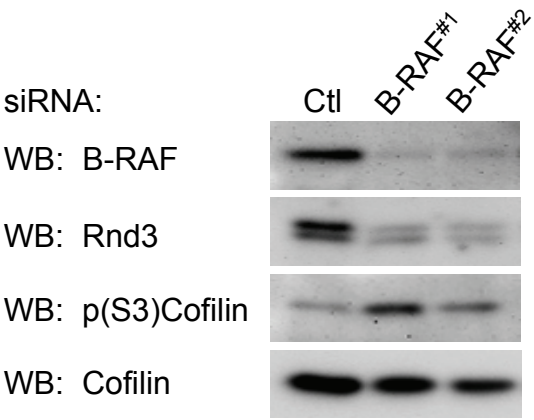
B-RAF#1 siRNA
B-RAF#2 siRNA

C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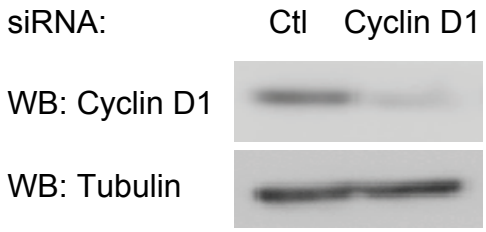
Ctl
B-RAF#1
B-RAF#2
WB: B-RAF
WB: Rnd3
WB: p(S3)Cofilin
WB: Cofilin

A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Ctl
Cyclin D1
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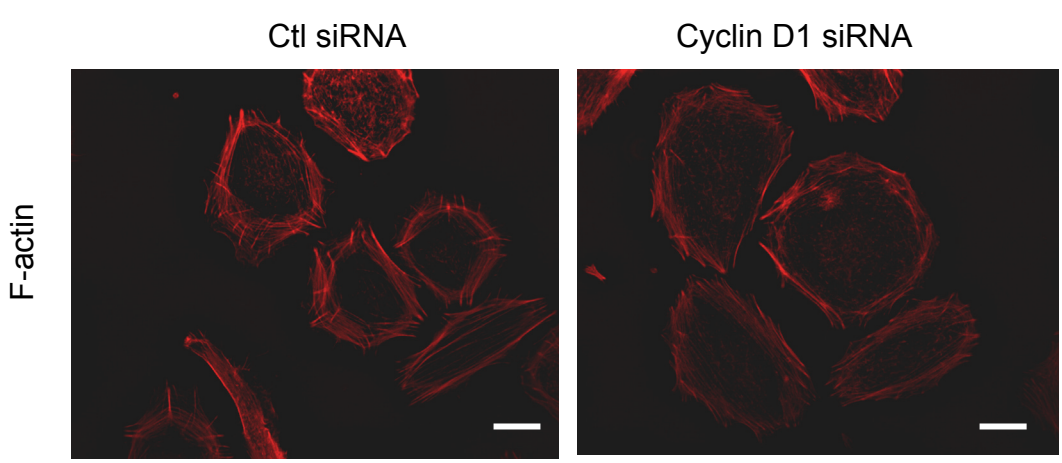
WB: Tubulin

B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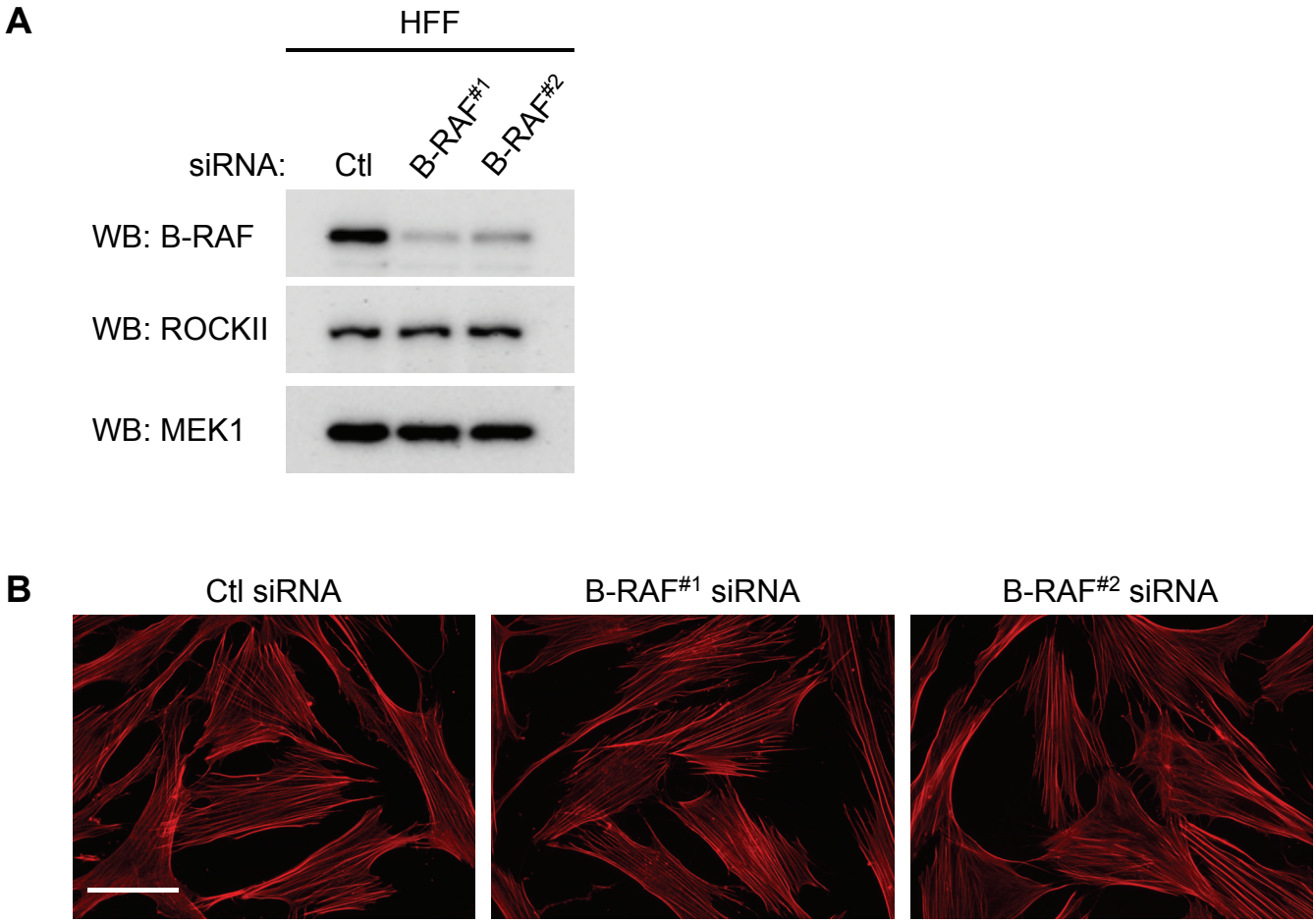
Cyclin D1 siRNA
F-actin


A
HFF
siRNA:
Ctl
B-RAF#1
B-RAF#2
WB: B-RAF
WB: ROCKII
WB: MEK1
B
Ctl siRNA
B-RAF#1 siRNA
B-RAF#2 siRNA