

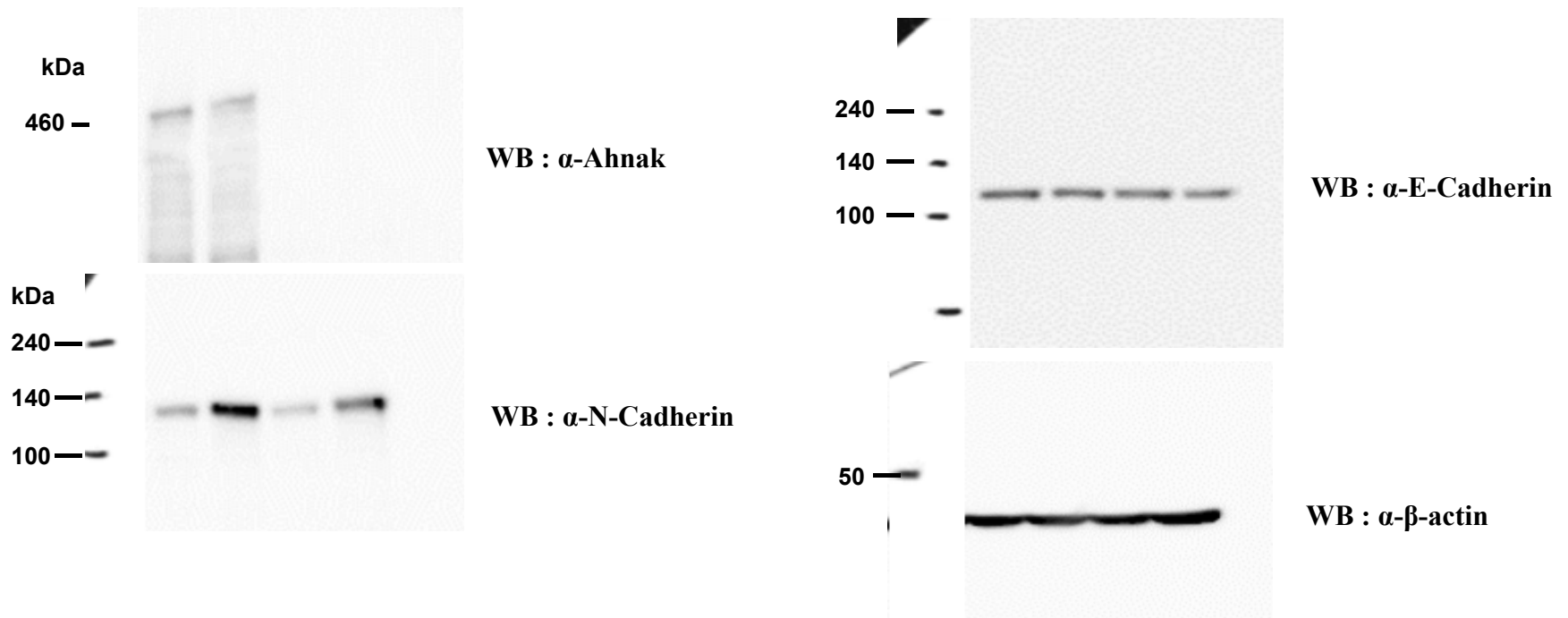
Ahnak promotes tumor metastasis through transforming growth factor- β -mediated epithelial-mesenchymal transition

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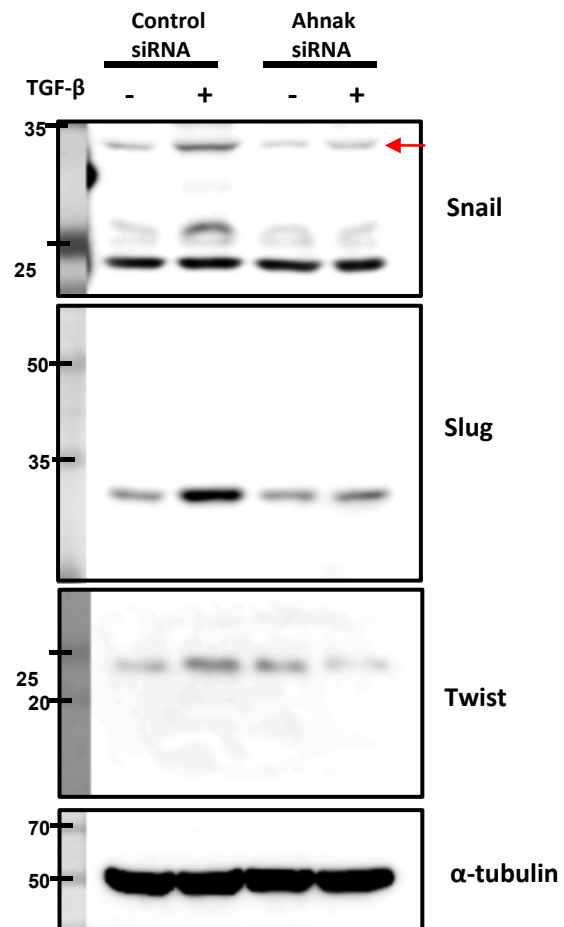
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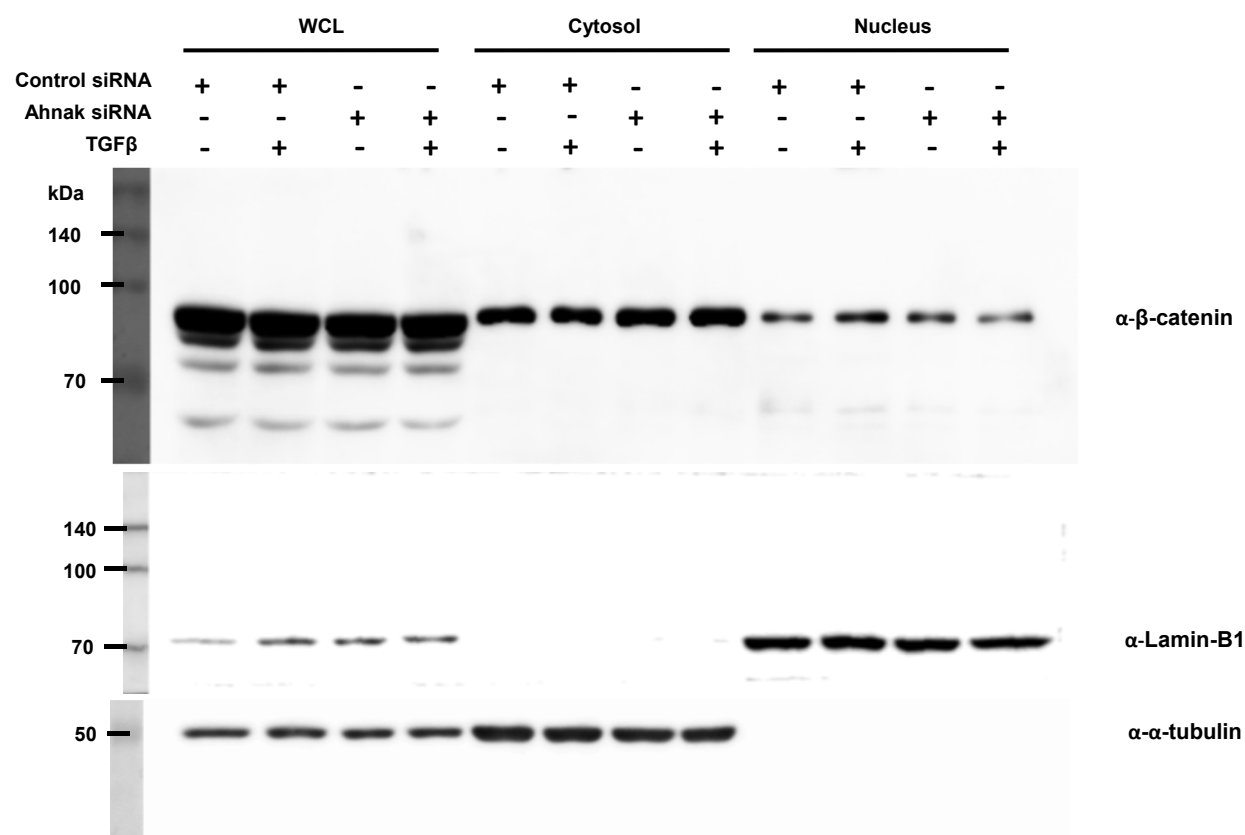
Supplementary Figure S1. Requirement of Ahnak for TGF β -induced EMT

HaCaT cells were transfected, serum deprived, and stimulated with TGF β (5 ng/mL) for 72 h. Cells were lysed and subjected to western blot (WB) analysis with the indicated antibodies.



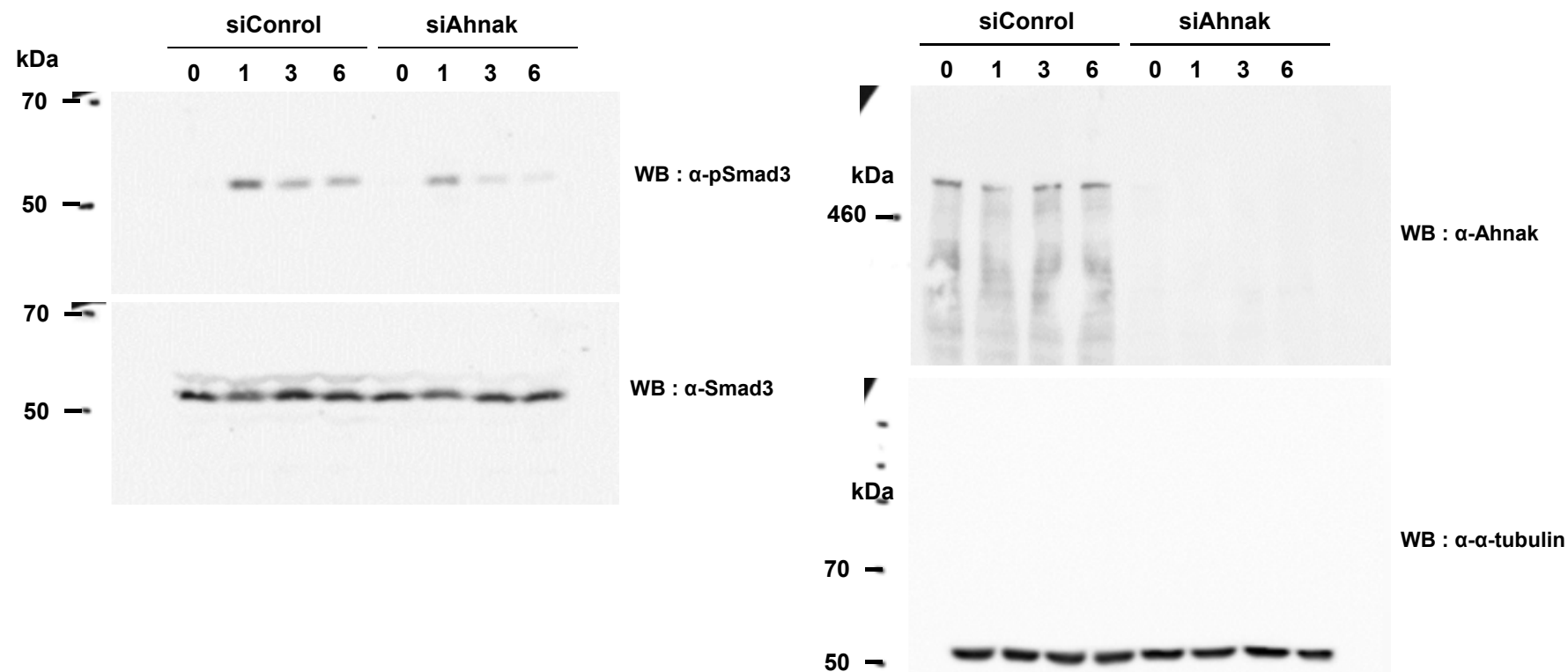
Supplementary Figure S2. TGF β -induced EMT markers expression in knockdown expression of Ahnak in HaCaT cells

HaCaT cells were transfected, serum deprived, and stimulated with TGF β (5 ng/mL) for 24 h. Cells were lysed and subjected to western blot (WB) analysis with the indicated antibodies.



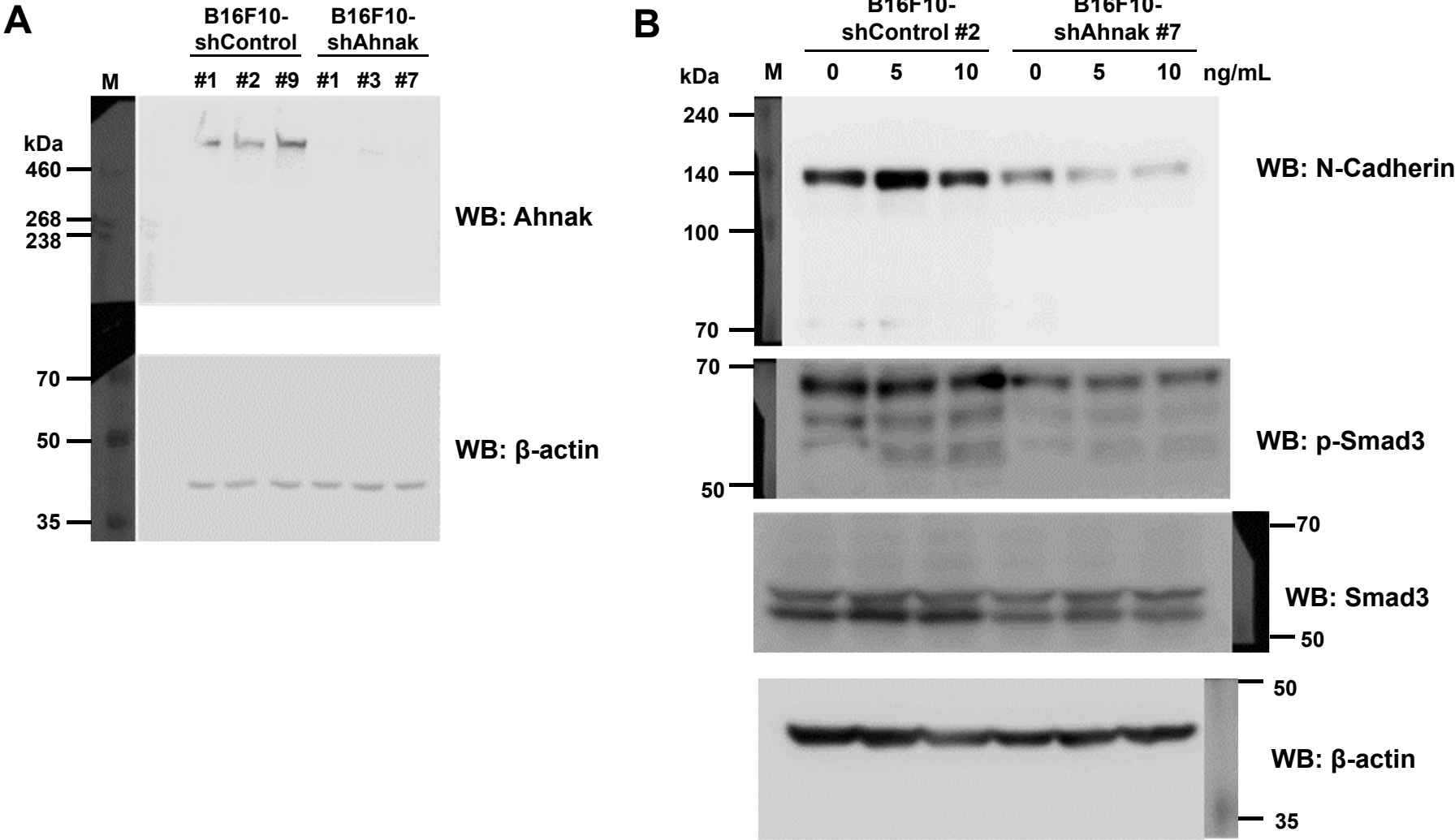
Supplementary Figure S3. Effect of Ahnak on TGF β -induced β -catenin nuclear localization in HaCaT cells

HaCaT cells (6×10^4) cultured in 100mm culture dishes were transfected control siRNA or Ahnak siRNA as described above. HaCaT cells were serum starved for 16h and then incubated with or without TGF- β (5ng/ml) for 1h. The nuclear fraction was isolated by the cell fractionation kit (Cell signaling; #9038) according to manufacturer's instruction.



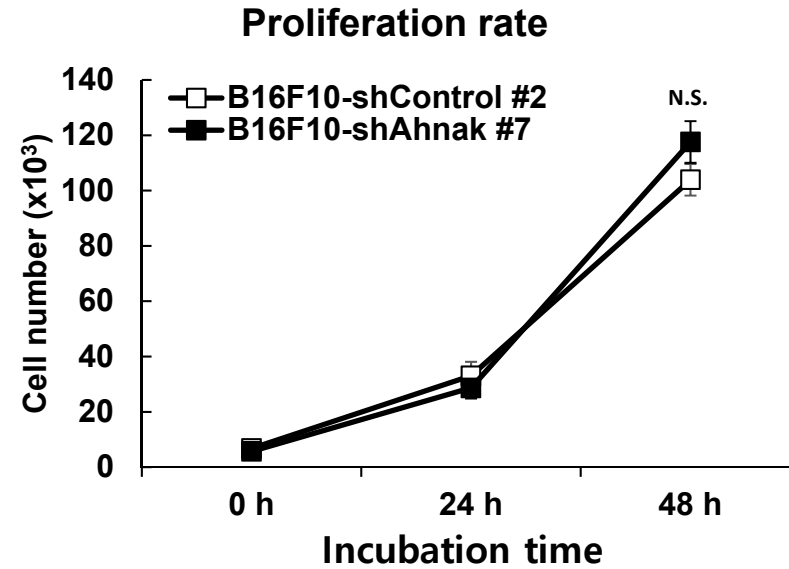
Supplementary Figure S4. Activation of TGF β /Smad3 signaling by Ahnak

HaCaT cells were transfected with the indicated siRNA, serum starved, and stimulated with TGF β (5 ng/mL) for the indicated time. Cells were lysed and subjected to western blot (WB) analysis with antibodies to p-Smad3, Smad3, Ahnak and actin.



Supplementary Figure S5. Ahnak regulates EMT-mediated melanoma cell migration and invasion

(A) B16F10 melanoma cells were stably infected with pGIPZ lentivirus-shControl and shAhnak and selected by colony picking to generate monoclonal cell line. Each cell line was subjected to western blot (WB) analysis with the indicated antibodies. (B) Ahnak-depleted B16F10 stable cells were serum starved and stimulated with TGF β (5 ng/mL) for 24 h. Cells were lysed and subjected to western blot (WB) analysis with the indicated antibodies.



Supplementary Figure S6. Proliferation rate of B16F10-shControl #2 and B16F10-shAhnak #7 cells

Both cell lines (B16F10-shControl #2 and B16F10-shAhnak #7 cells) were cultured in 96well plates for the indicated incubation time. The cells were harvested and counted. The data are means \pm SEM of three independent experiments.