SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Immunohistochemical analysis of TAK1 skin wound biopsy**. (A)** Negative control. Immunohistochemical staining of Day 5 mouse wound biopsies with pre-immune IgG as negative control. The section was processed as described in Figure 1B. Representative picture from wound epithelium and the adjacent wound bed was shown. E: epidermis; WB: wound bed; Scale bar 20 μm. **(B)** Relative expression level of TAK1 mRNA in normal human skin and biopsies from chronic non healing ulcers as determined by qPCR. Cyclophilin was used as a normalizing housekeeping gene. Statistical analysis was determined using two-tailed Mann-Whitney test, *** denotes $p<0.001$.

Figure S2. TAK1-deficient keratinocytes migrate faster, independent of cell proliferation. **(A)** Time-lapsed images of wounded cultures of control (K_{CTRL}) and TAK1-knockdown ($K_{TAK1\text{-}B}$) treated with mitomycin C (2 μg/ml). Scale bar 100 μm. See videos S3-4. Higher magnification images from video microscopy showing migratory front of K_{CTRL} and $K_{TAK1\text{-}B}$ (bottom two panels). Arrows indicate the focal adhesion points and microspike-like extensions in lamellipodia during migration. Scale bar 20 μm.

Figure S3. TAK1 deficiency enhances cell migration. Immunoblot analysis of active cdc42, Rac1 and RhoA in K_{CTRL} and $K_{TAK1\text{-}B}$ exposed to serum for the indicated time periods (min). Values below the blots represent the mean fold change in active cdc42, Rac1 and RhoA relative to level at zero min of K_{CTRL} (n=5). Representative pictures of immunoblot were shown.

Figure S4. TAK1 plays a homeostatic role in epidermal proliferation. Immunofluorescence staining of K_{CTRL} and $K_{TAK1\text{-}B}$-derived organotypic skin cultures (OTCs). Keratin 10 (CK 10) and involucrin (INV) were late and terminal epidermal differentiation, respectively. Proliferating and apoptotic cells were identified using Ki67 antibody and TUNEL assay, respectively (white arrows). OTC sections were counterstained with DAPI (blue). Dotted line represents epidermal-dermal junction. F denotes the underlying fibroblasts-embedded collagen. White dotted lines indicate epidermal-dermal junction. H&E: Haematoxylin and eosin staining. Scale bar 40 μm. Mean proliferating and apoptotic cells were numerated from 3 standardized microscopic fields per section, performed on 3 sections from 4 independent OTC constructions (n=36).

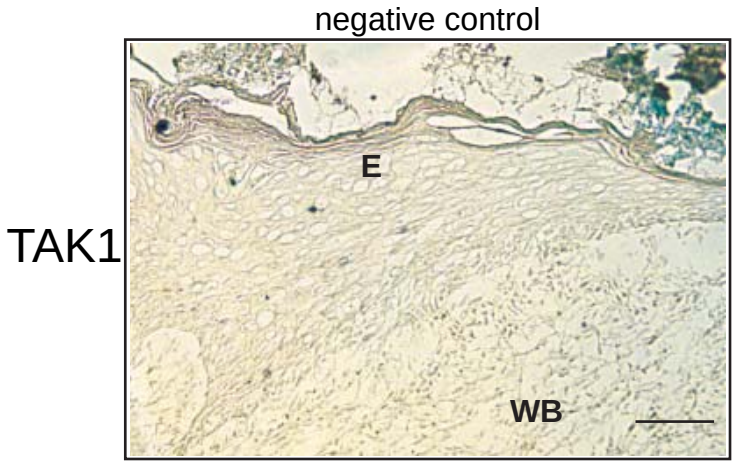
Figure S5. TAK1 regulates epidermal proliferation via a double paracrine signaling involving PDGF-B. **(A)** Immunofluorescence staining of K_{CTRL} and $K_{TAK1\text{-}B}$-derived OTCs cultured with only collagen (COL). In the absence of underlying fibroblasts, no difference in cell proliferation index was observed between K_{CTRL} and $K_{TAK1\text{-}B}$ epidermis. Proliferating cells were identified using Ki67 antibody (white arrows). OTC sections were counterstained with DAPI (blue). White dotted lines indicate epidermal-dermal junction. **(B)** Human Growth Factor Antibody Array I (RayBio®): Protein analysis of conditioned medium from two-weeks old K_{CTRL} and $K_{TAK1\text{-}B}$-derived OTCs. Spots corresponding to HGF, PDGF-BB and TGF-β were boxed. Representative pictures of array were shown. **(C)** Immunofluorescence staining of K_{CTRL}- and $K_{TAK1\text{-}B}$-derived OTCs cultured with underlying fibroblasts (F) in the presence either vehicle (PBS) or neutralizing anti-PDGF- antibody. Blocking the activity of PDGF negates the pro-mitogenic effect of TAK1 deficiency in keratinocytes. Dotted white line represents epidermal-dermal junction. H&E: Haematoxylin and eosin staining. Scale bar 40 μm. Mean proliferating cells was numerated from 3 standardized microscopic fields per section, performed on 3 sections from 3 independent OTC constructions (n=27).

Figure S6. Ectopic expression of pVHL in $K_{TAK1\text{-}B}$ inhibits Sp1 binding to the promoters of PDGF-B, integrins β1 and β5. Chromatin from vector- or pVHL-transfected $K_{TAK1\text{-}B}$ was immunoprecipitated with either an anti-Sp1 antibody (Ab) or preimmume IgG (pre). Enrichment of a DNA fragment encompassing the Sp1 binding sites was evaluated by PCR. Aliquots of the extracts were

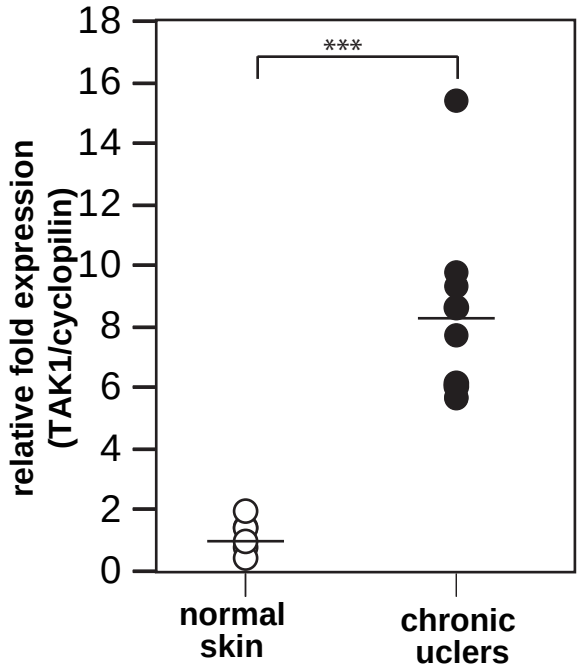
also used before immunoprecipitation (input). Two stable $K_{TAK1\text{-}B}$ clones expressing pVHL were used. No amplified signal was obtained in using pre-immune IgG. A control region upstream of NF-κB binding site served as negative control.

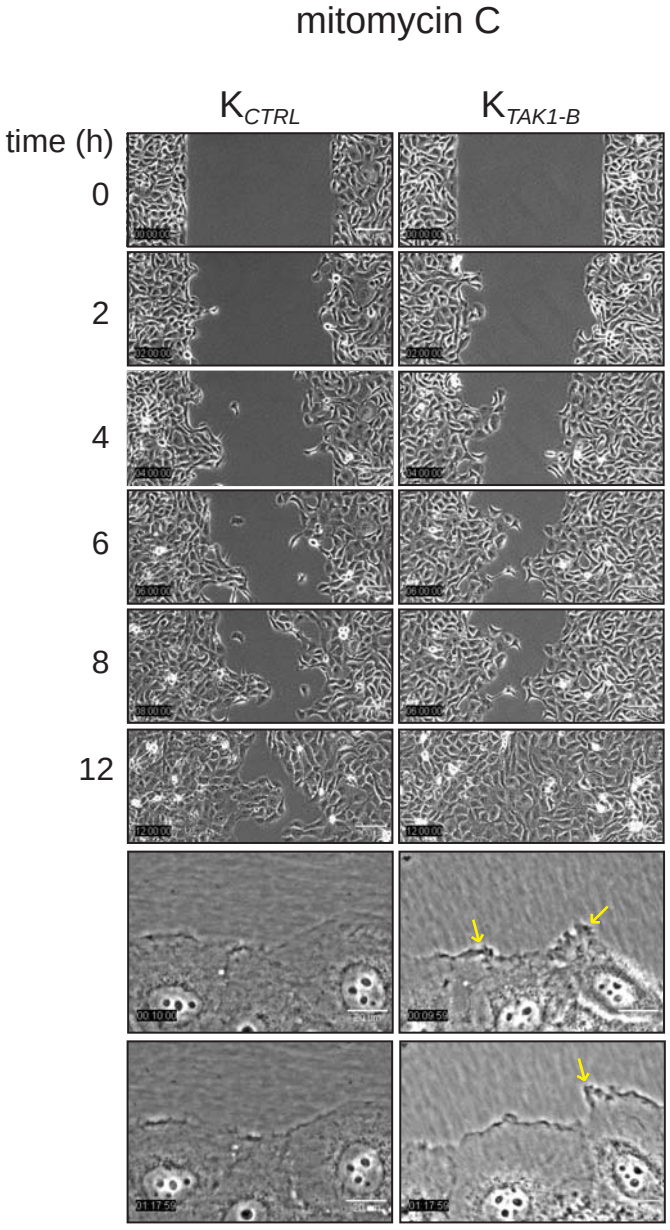
Figure S7. pVHL suppresses migration and proliferation via a HIFα-independent fashion. **(A)** Immunoblot analysis of HIFα on K_{CTRL}, $K_{TAK1\text{-}B}$ and TAK1-transfected $K_{TAK1\text{-}B}$ epidermis (left panel). β-tubulin showed equal loading and transfer. Time-lapsed images of wounded cultures in cobalt chloride-simulated (50 mM) hypoxia condition. See videos S8. Scale bar 100 μm. **(B)** Immunohistochemical analysis of pVHL skin wound biopsy**.** Immunohistochemical staining of Day 5 mouse wound biopsies with either pre-immune IgG as negative control (left panel) or with anti-pVHL antibody (right panel). The section was processed as described in Figure 1B. DAB with nickel (dark blue) was used as substrate. Representative picture from wound epithelium and the adjacent wound bed was shown. Scale bar 20 μm.

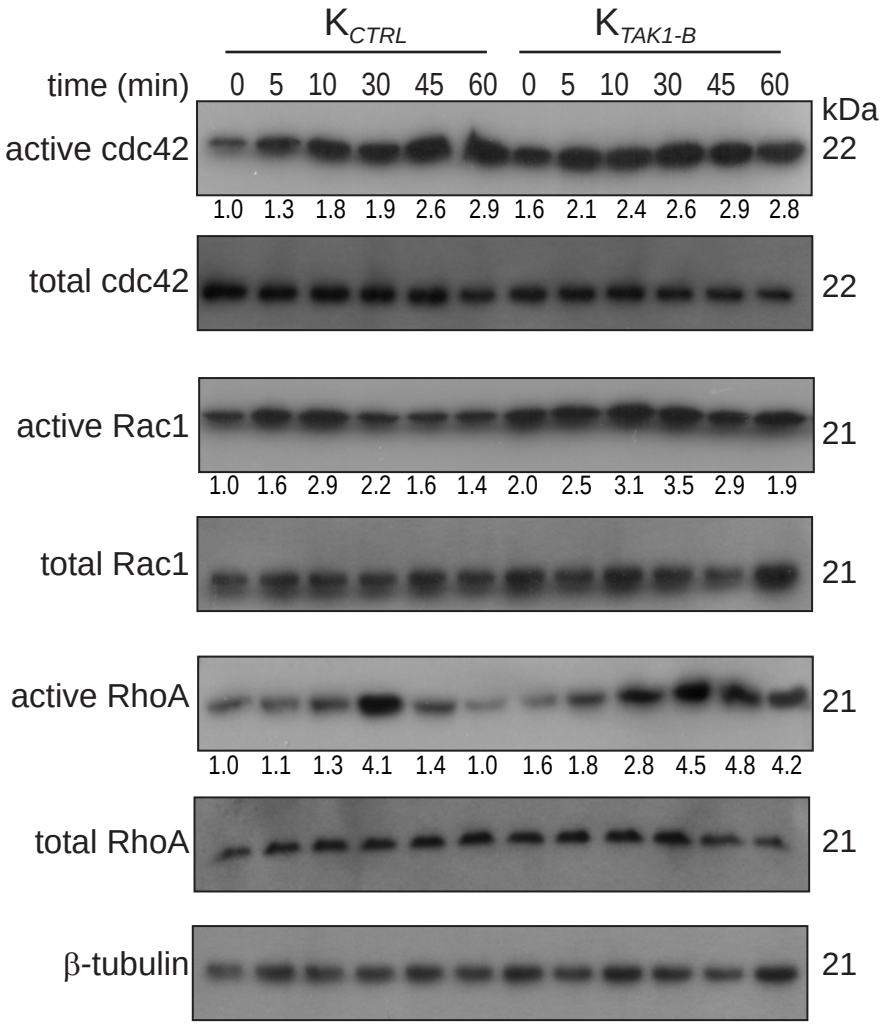
A


negative control
TAK1
E
WB

B


relative fold expression
(TAK1/cyclopilin)
18
16
14
12
10
8
6
4
2
0
***
normal
skin
chronic
uclers


mitomycin C
K_{CTRL}
K_{TAK1-B}
time (h)
0
2
4
6
8
12


K_CTRL
K_TAK1-B
time (min)
0 5 10 30 45 60 0 5 10 30 45 60
kDa
active cdc42
22
1.0 1.3 1.8 1.9 2.6 2.9 1.6 2.1 2.4 2.6 2.9 2.8
total cdc42
22
active Rac1
21
1.0 1.6 2.9 2.2 1.6 1.4 2.0 2.5 3.1 3.5 2.9 1.9
total Rac1
21
active RhoA
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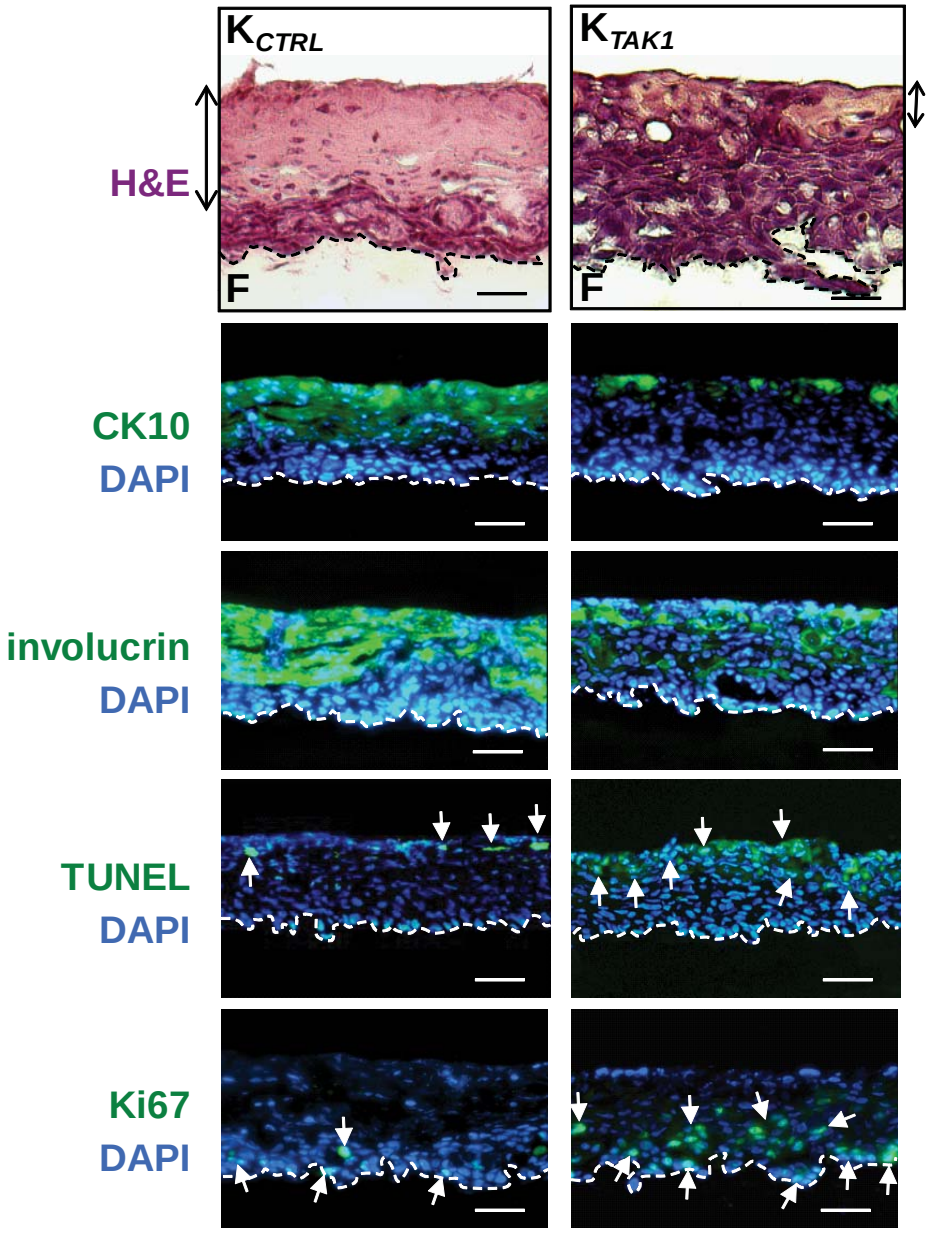
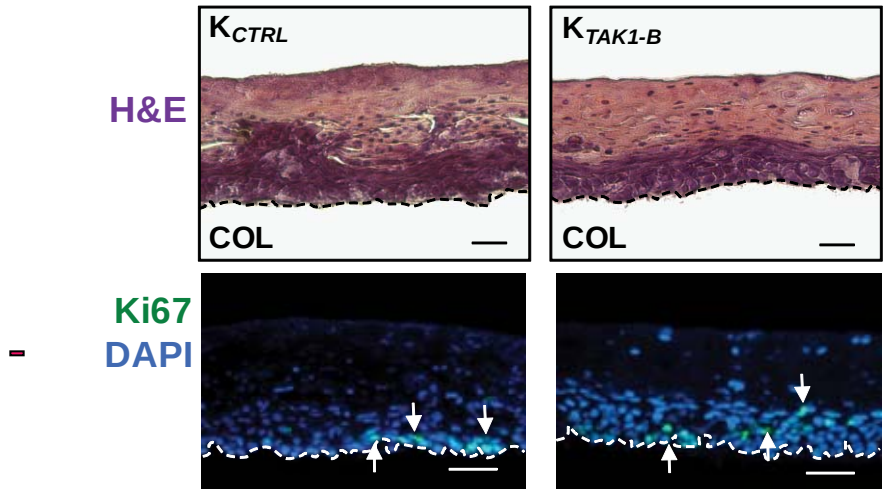
total RhoA
21
β-tubulin
21


K CTRL
K TAK1
H&E
F
F
CK10
DAPI
involucrin
DAPI
TUNEL
DAPI
Ki67
DAPI

A

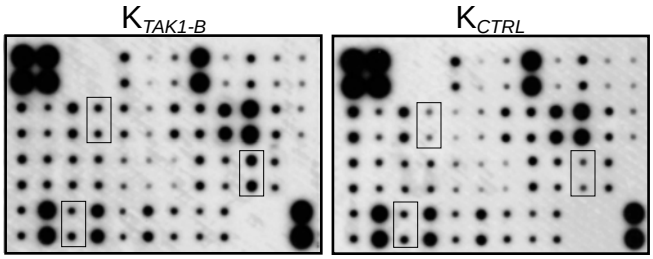


B

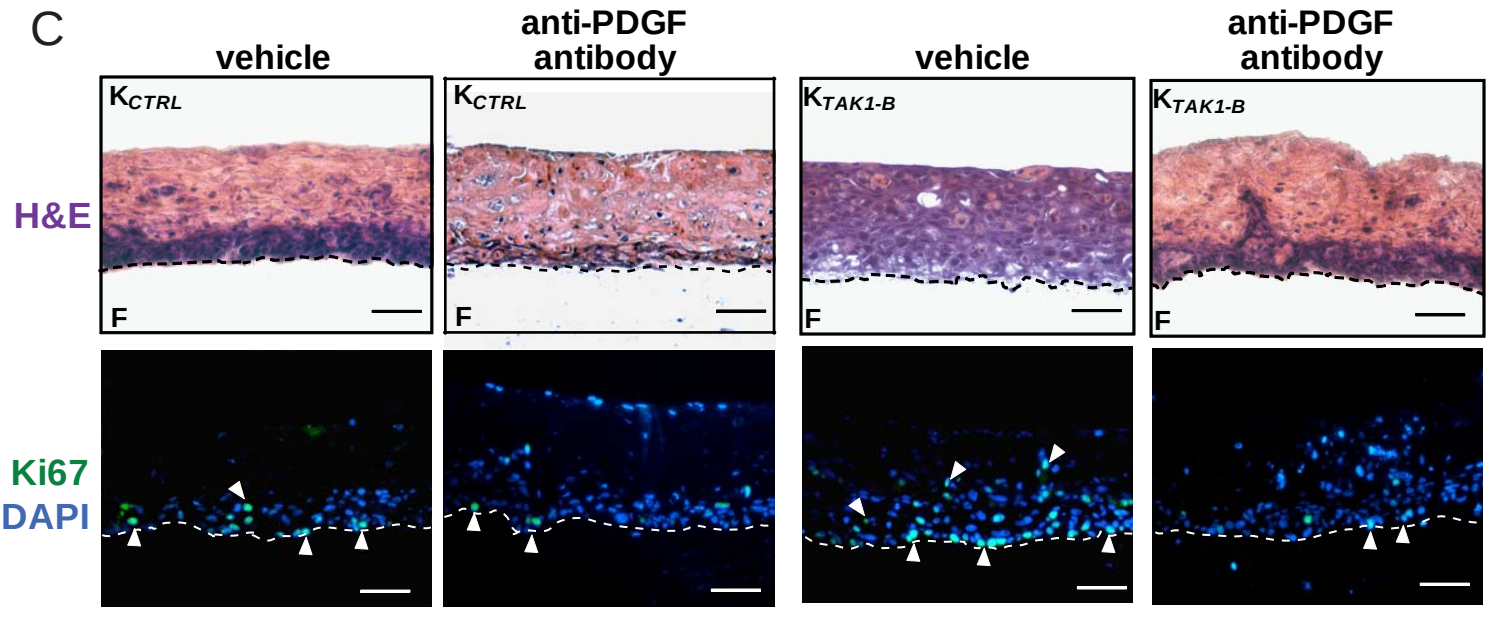
Human Growth Factor Antibody Array I Map

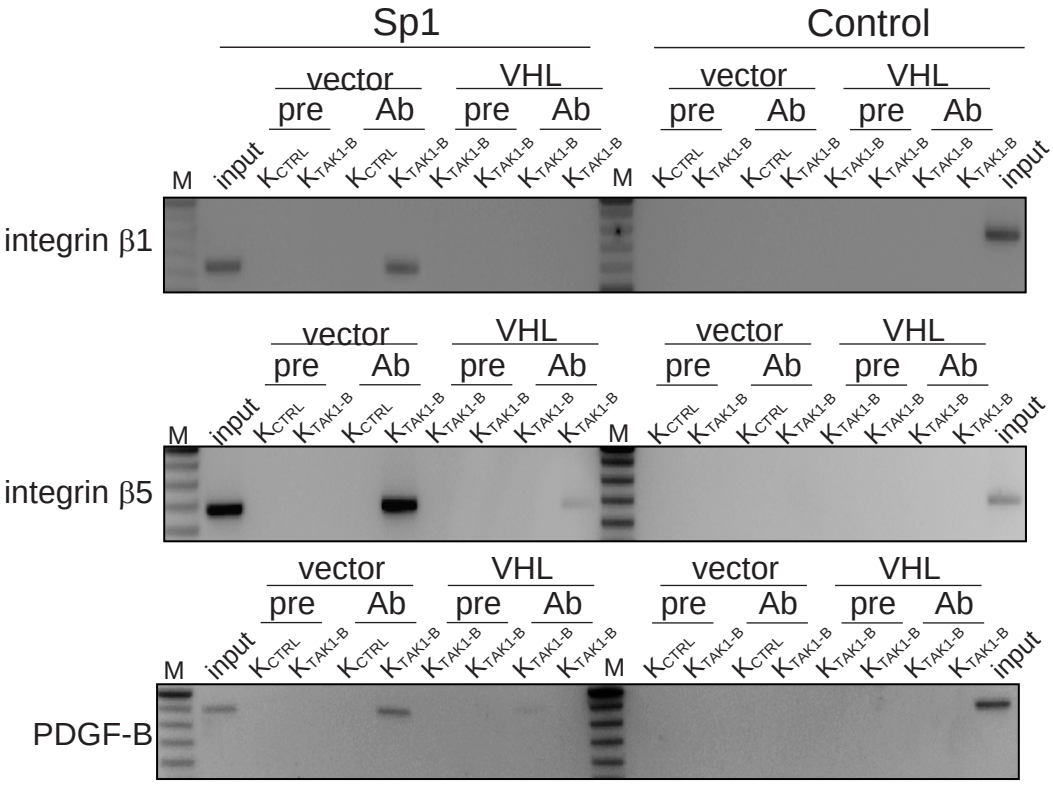
	A	B	C	D	E	F	G	H	I	J	K	L
1	POS	POS	NEG	NEG	AR	bFGF	b-NGF	EGF	EGF R	FGF-4	FGF-6	FGF-7
2	POS	POS	NEG	NEG	AR	bFGF	b-NGF	EGF	EGF R	FGF-4	FGF-6	FGF-7
3	GCSF	GDNF	GM-CSF	HB-EGF	HGF	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-6	IGF-I	IGF-I SR
4	GCSF	GDNF	GM-CSF	HB-EGF	HGF	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-6	IGF-I	IGF-I SR
5	IGF-II	M-CSF	M-CSF R	NT-3	NT-4	PDGF R α	PDGF R β	PDGF-AA	PDGF-AB	PDGF-BB	PIGF	SCF
6	IGF-II	M-CSF	M-CSF R	NT-3	NT-4	PDGF R α	PDGF R β	PDGF-AA	PDGF-AB	PDGF-BB	PIGF	SCF
7	SCF R	TGF-α	TGF-β	TGF-β 2	TGF-β 3	VEGF	VEGF R2	VEGF R3	VEGF-D	BLANK	BLANK	POS
8	SCF R	TGF-α	TGF-β	TGF-β 2	TGF-β 3	VEGF	VEGF R2	VEGF R3	VEGF-D	BLANK	BLANK	POS

* For use with serum, plasma, condition medium, urine and all other body fluids, cell lysates and certain tissue lysates samples

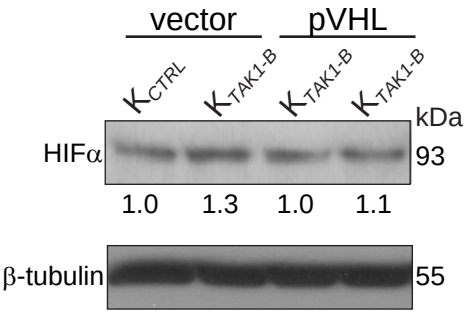


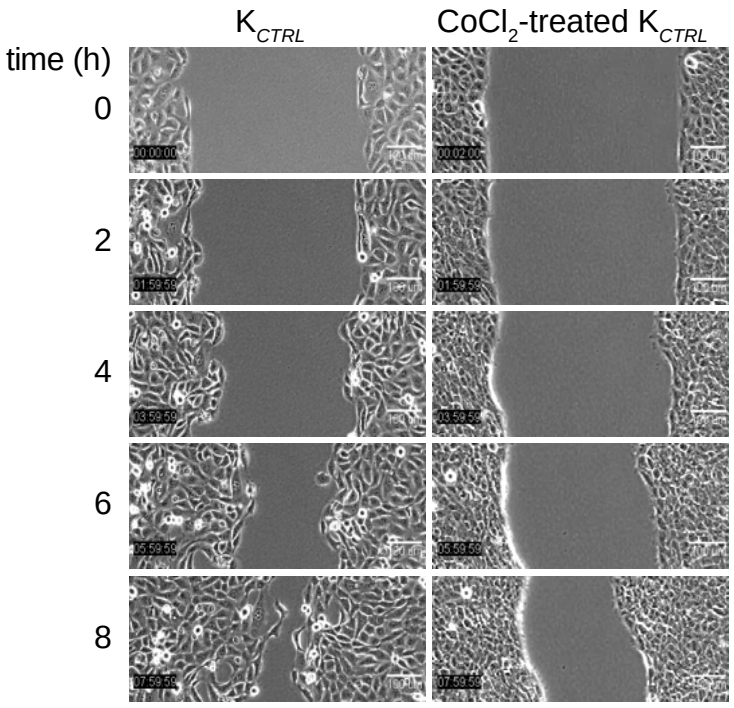
C



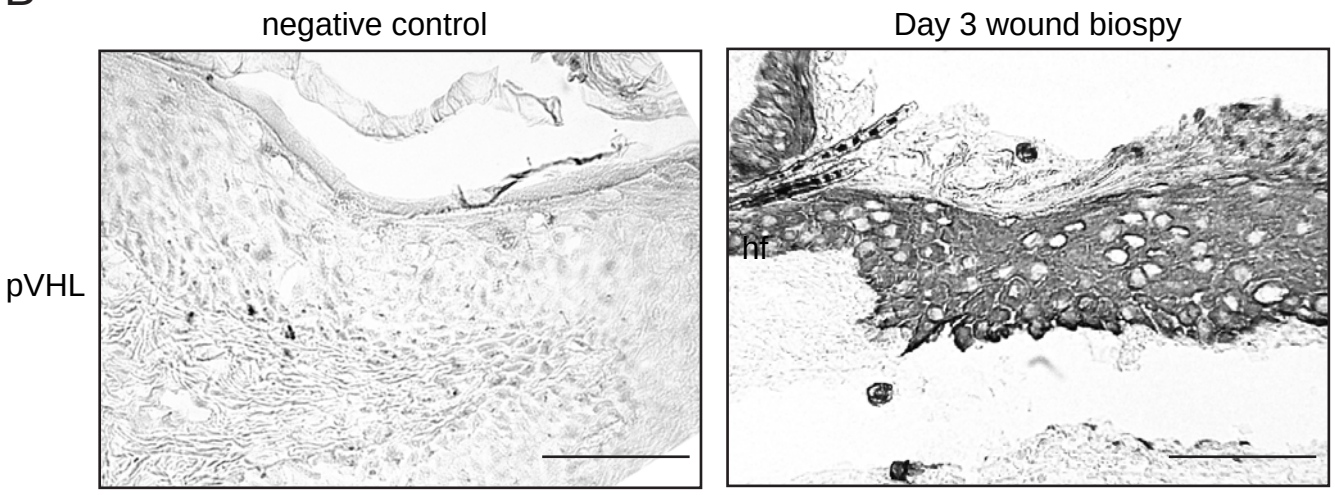

Sp1
Control
vector
VHL
pre
Ab
M
input
K_{CTRL}
$K_{TAK1\text{-}B}$
integrin β1
integrin β5
PDGF-B

A


vector
pVHL
K_{CTRL}
K_{TAK1-B}
K_{TAK1-B}
K_{TAK1-B}
kDa
HIFα
93
1.0
1.3
1.0
1.1
β-tubulin
55


K_{CTRL}
$CoCl_2$-treated K_{CTRL}
time (h)
0
2
4
6
8

B


negative control
Day 3 wound biospy
pVHL
hf

SUPPLEMENTAL TABLE

Table S1. Oligonucleotide sequences

Primer pairs (S: sense; AS: anti-sense)	Sequence (5'-3')
Actin S	CTTCTCTCTAAGGAGAATGGCCCAG
Actin AS	CAACTGGTCTCAAGTCAGTGTACAGG
Actin RT1	ACGTGGACATCCGCAAAGAC
Actin RT2	CGCTCAGGAGGAGCAATGAT
HGF S	TCCCTTCTCGAGACTTGAAAGAT
HGF AS	CTGCATTTCTCATTTCCCATT
PDGFB S	TGAACATGACCCGCTCCCACTC
PDGFB AS	TGCCGTCTTGTCATGCGTGTG
PDGFA S	CGGATACCTCGCCCATGTTCTG
PDGFA AS	TCCGGATTCAGGCTTGTGGTCG
TAK A1	AAAGAAGAGGTTGTTGGAAGAGGAG
TAK A2	AAAACTCCTCTTCCAACAACCTCTT
TAK B1	AAAGTGGCTTATCTTACACTGGA
TAK B2	AAAATCCAGTGTAAGATAAGCCA
TAK1 RT1	GTTCCGTGTAAGGGCTTTGA
TAK1 RT2	GAGCAGCTGCCACTTACCTTTACA
U6	GCTTACCGTAACTTGAAAGTATTTCG
H1	CTGGGAAATCACCATAAACGTGAA
pVHL promoter S	GATCCTCGAGCGTGATGATTGGGTGTTCCC
pVHL promoter AS	GATCAAGCTTCGACGCCTGCCTCCTCCGCG
chIP primers (S, AS)	
ITGB1SP1_S	TCAGTGGACAAACGGGAGCGAG
ITGB1SP1_AS	CTCTCCCTCCGGAAACGCATTC
ITGB1control_S	TCGACATGCGTTCAGCCATTC

ITGB1control_AS	GGAGTGATAATGTGCTTGCCTG
ITGB3SP1_S	GGCGAGAGAGGAGCAATAG
ITGB3SP1_AS	GAGCCTCACTCACCTCCTAC
ITGB3control_S	TTGGAGCAGGATGAGGCTTTGC
ITGB3control_AS	CCCAGTACCAAAGAGGCCACTC
ITGB5SP1_S	CATTTCCTTCCCTCCTTC
ITGB5SP1_AS	TGTGAATTGAGTTTGTTGC
ITGB5control_S	GAGAGACAGCCCAGCATCTCAG
ITGB5control_AS	CACGCACACTGTGGACTTCACC
PDGFBSP1_S	TCCGGGCCAGAAGAGGAAAG
PDGFBSP1_AS	CCCATCTTTGATCCTTCTG
PDGFBcontrol_S	GGTGAATCAGAGTGGAGTATG
PDGFBcontrol_AS	CTGTGGACTGTCCTGAGGAG
Mutagenesis primers	
VHL mutant S	GTGGATCGCGGAGGGAgatCtCCGGAGGGCGGAGAAC
VHL mutant AS	GTTCTCCGCCCTCCGGAGATCTCCCTCCGCGATCCAC

Underlined nucleotides indicate ‘sticky-ends’ that facilitate ligation with the *Bbs*I-digested siRNA expression vector.

SUPPLEMENTAL VIDEO LEGENDS

Videos. *In vitro* scratch wound assay performed using (**S1)** control keratinocytes (K_{CTRL}) and (**S2**) TAK1-deficient keratinocytes (K_{TAK1-B}). Cell migration was recorded at 2 min interval for 9 h. Scale bar 100 μm.

Videos. *In vitro* scratch wound assay performed using (**S3)** control keratinocytes (K_{CTRL}) and (**S4**) TAK1-deficient keratinocytes (K_{TAK1-B}) in the presence of 2 μg/ml of mitomycin C. Cell migration was recorded at 2 min interval for 9 h. Scale bar 100 μm.

Videos. Migratory front cell phenotype of (**S5**) K_{CTRL} and (**S6**) K_{TAK1-B}. Cell migration was recorded at 1 min interval for 2 h. Scale bar 20 μm.

Video S7. *In vitro* scratch wound assay performed using TAK1-transfected K_{TAK1-B}. Cell migration was recorded at 2 min interval for 9 h. Scale bar 100 μm.

Video S8. *In vitro* scratch wound assay performed using cobalt chloride-simulated hypoxia condition on control keratinocytes (K_{CTRL}). Cell migration was recorded at 2 min interval for 9 h. Scale bar 100 μm.