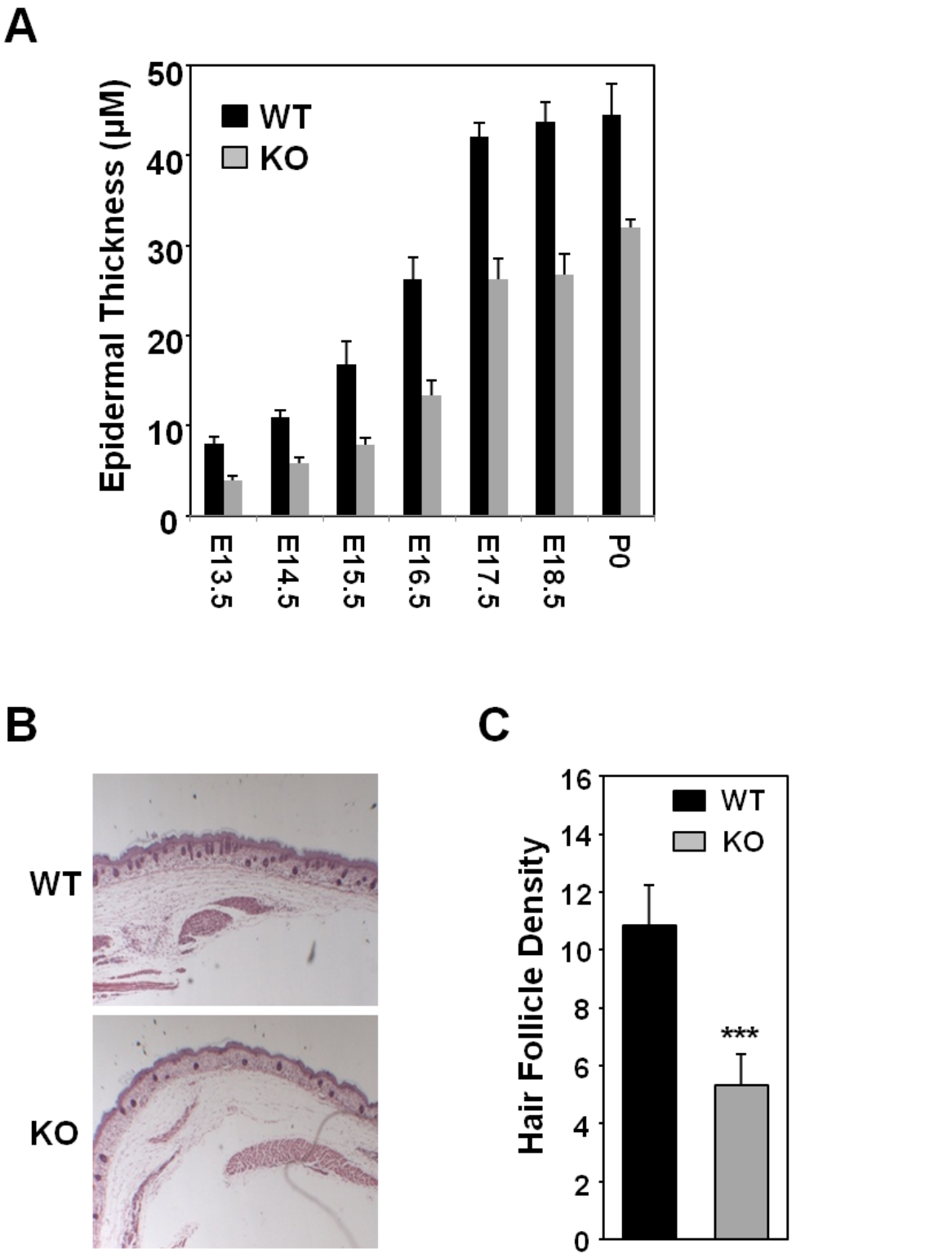

Fig. S1. Ctip2-null epidermis is hypotrophic during development. (A) Epidermal thickness was measured on Hematoxylin-Eosin-stained sections of dorsal skin collected from wild-type (WT) and Ctip2-null (KO) embryos at indicated embryonic days. **(B-C)** Decreased hair follicle density in Ctip2-null E18.5 epidermis. (B) Hematoxylin-Eosin-stained sections of dorsal skin biopsies from WT and KO embryos at E18.5. (C) Hair follicle density per field was counted on the Hematoxylin-Eosin-stained sections from E18.5 WT (n=3) and KO (n=3) dorsal skin. Ten fields per section (3 sections/slide) from each of the WT and KO embryos were measured. WT, black bars; KO, gray bars. The results are representative of at least three independent studies.

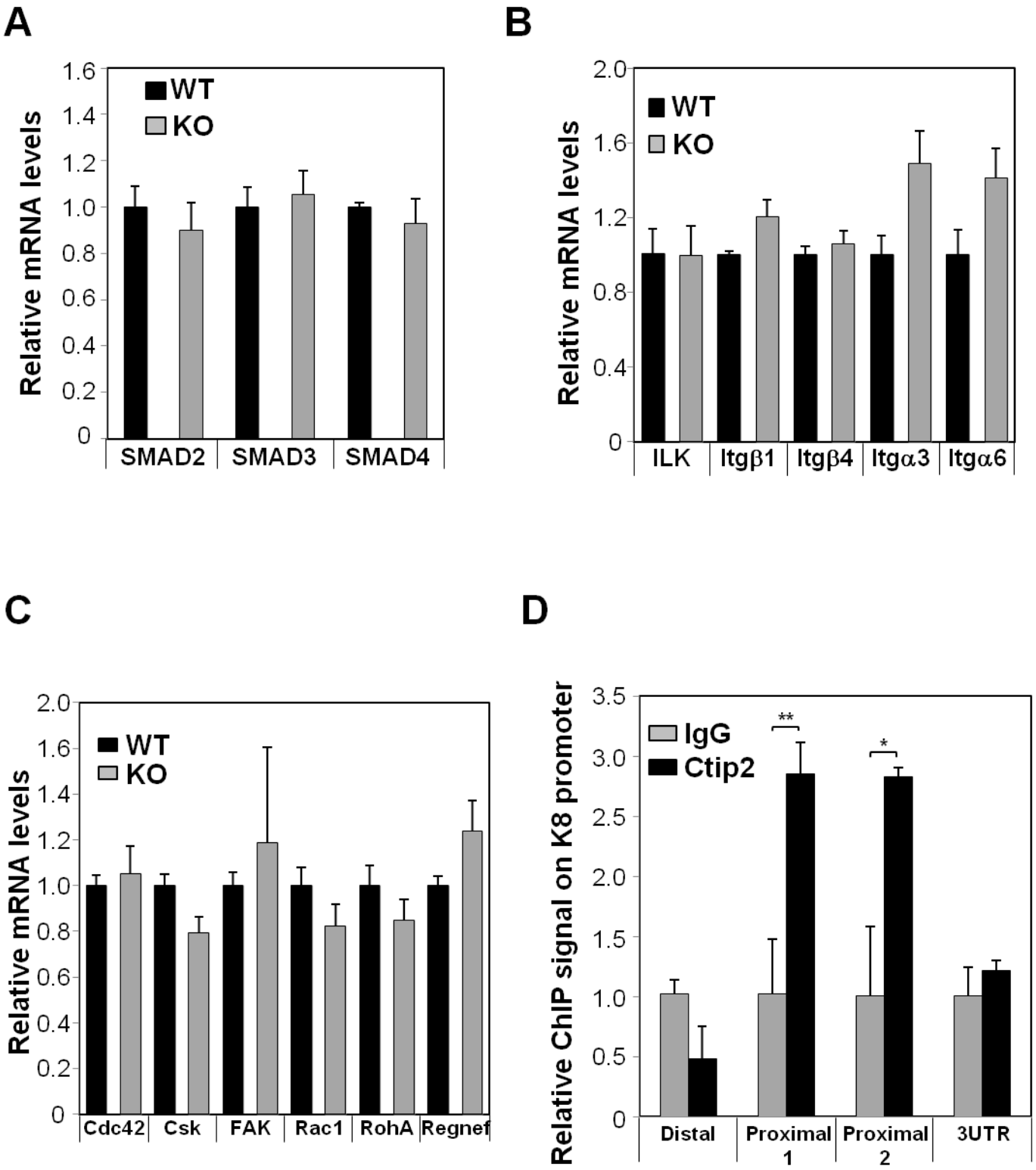

Fig. S2. The expression of genes regulating cytoskeletal organization or EMT in the absence of Ctip2. (A) Expression of SMAD family genes in Ctip2-null keratinocytes. Cultured keratinocytes were harvested for RT-qPCR analyses of expression of SMADs, including SMAD2, SMAD3 and SMAD4 as indicated. SMADs mRNA expression level was calculated relative to the expression of a house keeping gene, GAPDH. **(B-C)** Expression of genes regulating cytoskeletal organization is unaltered in Ctip2-null keratinocytes. Cultured keratinocytes were harvested from WT and Ctip2 KO keratinocytes for RT-qPCR analyses of integrin family genes, including ILK, Itgb1, Itgb4, Itga3 and Itga6 (B), as well as small GTPase related genes, including Cdc42, Csk, FAK, Rac1, RohA and Regnef (C). Relative mRNA expression of each gene was calculated relative to the expression of a house keeping gene, GAPDH. **(D)** Ctip2 is recruited to the promoter of keratin 8 (K8). Subconfluent cultured keratinocytes were subjected to chromatin immunoprecipitation (ChIP) analyses using anti-Ctip2 antibody. IgG (rat) was used as a control. The purified chromatin DNA was analyzed by qPCR using specific primers covering either the distal promoter, two proximal promoters or 3′UTR region of K8 as indicated. Each bar represents the relative ratio to IgG of each ChIP. Bars represent mean expression levels ± s.e.m. (n=3). WT, black bar; KO, gray bar. All experiments were performed in triplicates.

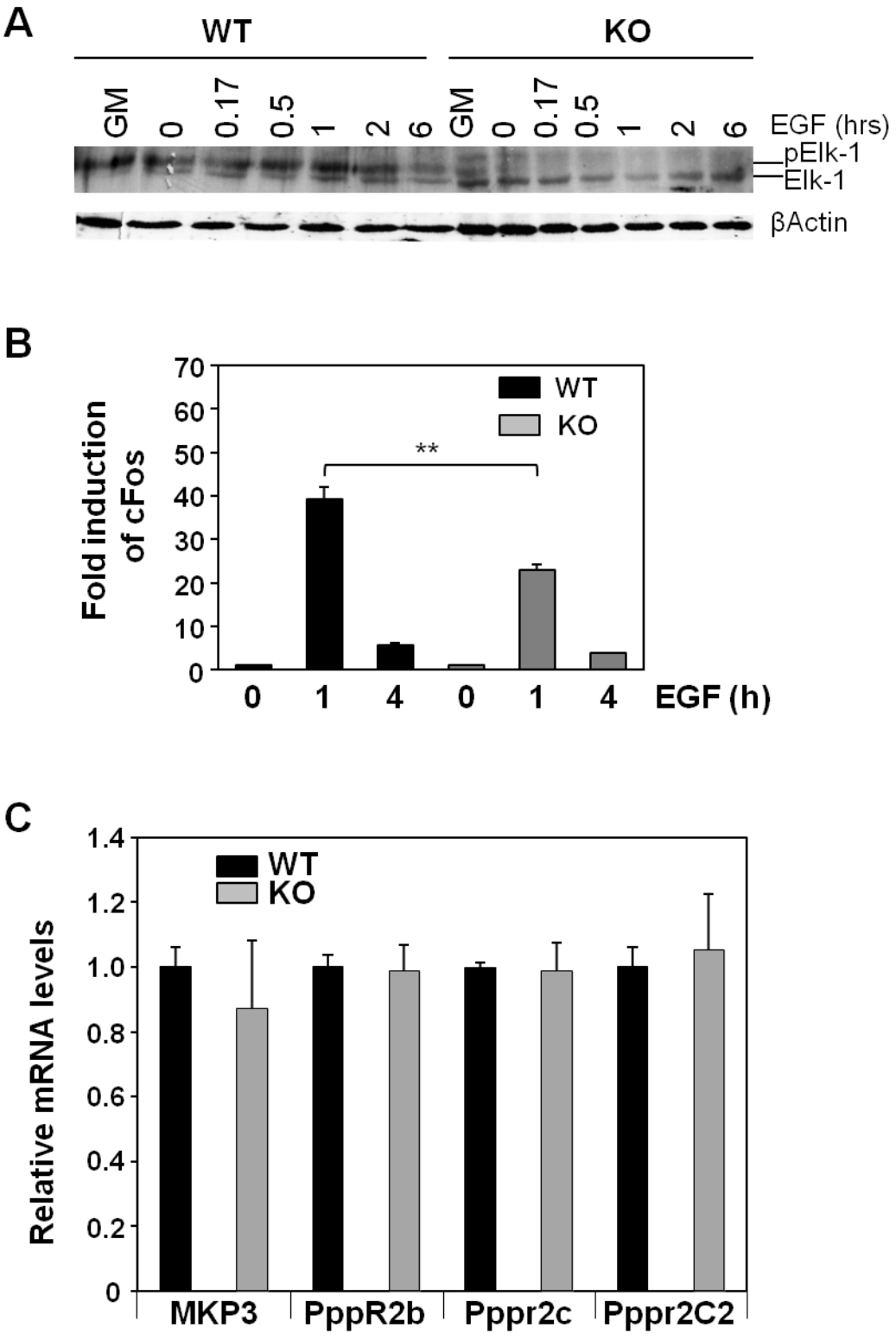

Fig. S3. Induction of Elk-1 phosphorylation and cFos expression by EGF treatment in cultured keratinocytes. (**A)** Impaired Elk-1 phosphorylation in Ctip2-null keratinocytes. Keratinocytes cultured under growth medium (GM) conditions were depleted of growth factors overnight then treated with 10 ng/ml EGF for indicated time before harvesting for immunoblotting analyses using indicated antibodies. Equal amounts of protein (10 μg) were loaded in each well. β-actin was used as a loading control. (**B)** Cultured WT and KO keratinocytes were depleted of growth factors overnight then treated with 10 ng/ml EGF for indicated hrs before harvest for RT-qPCR analyses of cFos mRNA expression (normalized to GAPDH). Results are shown as mean ± s.e.m. (n=3) of the relative cFos mRNA (**P<0.001). Statistical analyses were performed using the Student's un-paired t-test and GraphPad Prism software. **(C)** Expression of ERK phosphatases were unaltered in Ctip2-null keratinocytes. Cultured keratinocytes were harvested for RT-qPCR analyses of a group of ERK phosphatases, including MKP3, PppR2b, PppR2c and PppR2c2 as indicated. Relative mRNA expression of each gene was calculated relative to the expression of a house keeping gene, GAPDH. Bars represent mean expression levels ± s.e.m. (n=3). WT, black bar; KO, gray bar. The results are representative of at least three independent studies.

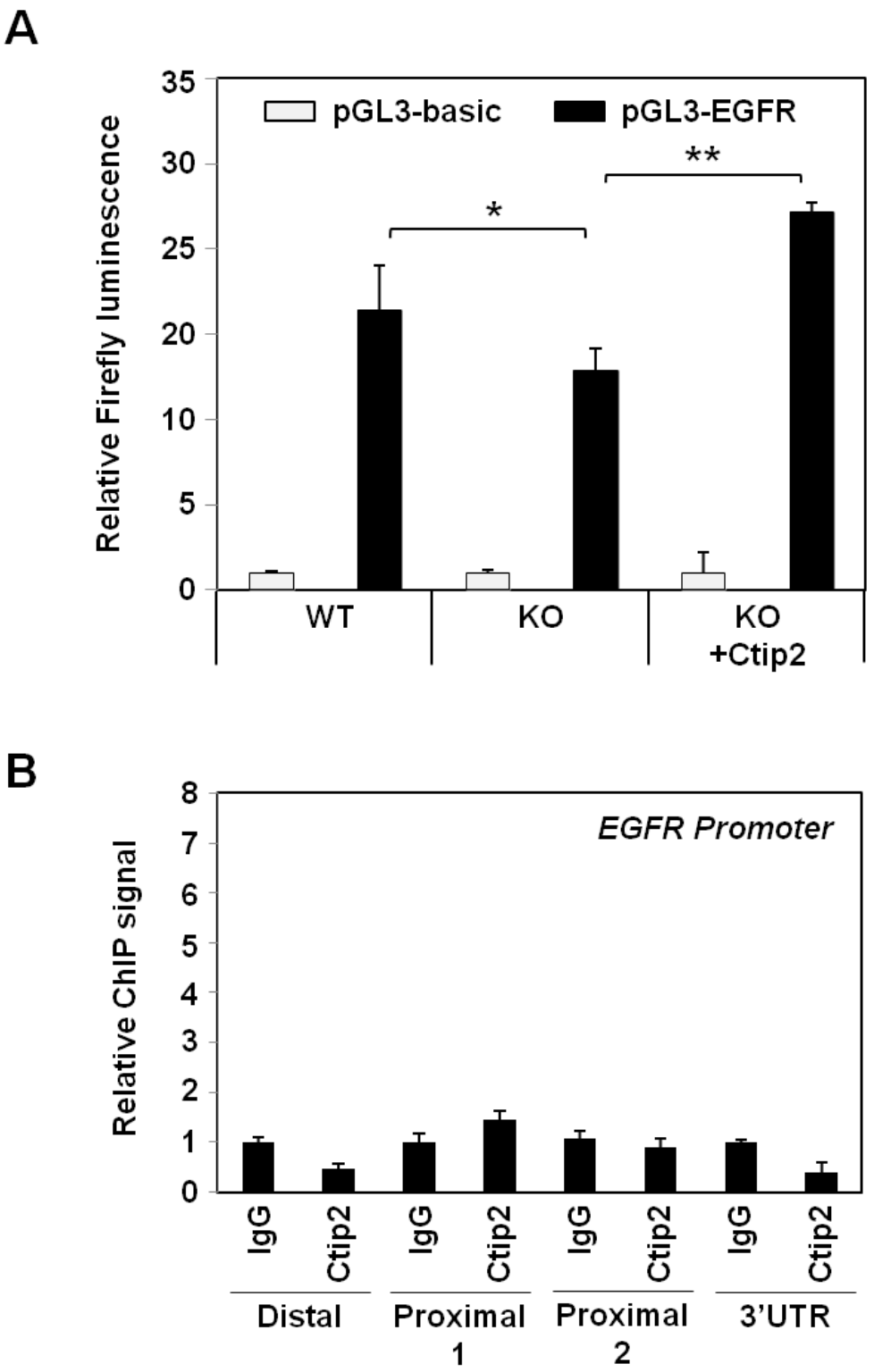

Fig. S4. Regulation of EGFR promoter by Ctip2. (A) Ctip2 positively regulates EGFR promoter in cultured keratinocytes. Wild-type (WT) and Ctip2-null (KO) keratinocytes were co-transfected with a promoterless luciferase construct (pGL3-basic) or luciferase reporter constructs bearing EGFR promoter with or without an expression vector encoding Ctip2 as indicated. Renilla luciferase construct driven by SV40 promoter was also co-transfected for normalization. Bars represent mean expression levels ± s.e.m. (n=3) of the relative fold expression of firefly luciferase. Statistical significance was determined by Student's un-paired *t*-test using the GraphPad Prism software (**P*<0.05; ***P*<0.01). **(B)** Negative controls for Ctip2 ChIP assays shown in Fig. 5F. ChIP assays were performed as described in Fig. 5F, except that they were performed using E16.5 epidermal keratinocytes from Ctip2 KO embryos. All experiments were performed in triplicates.

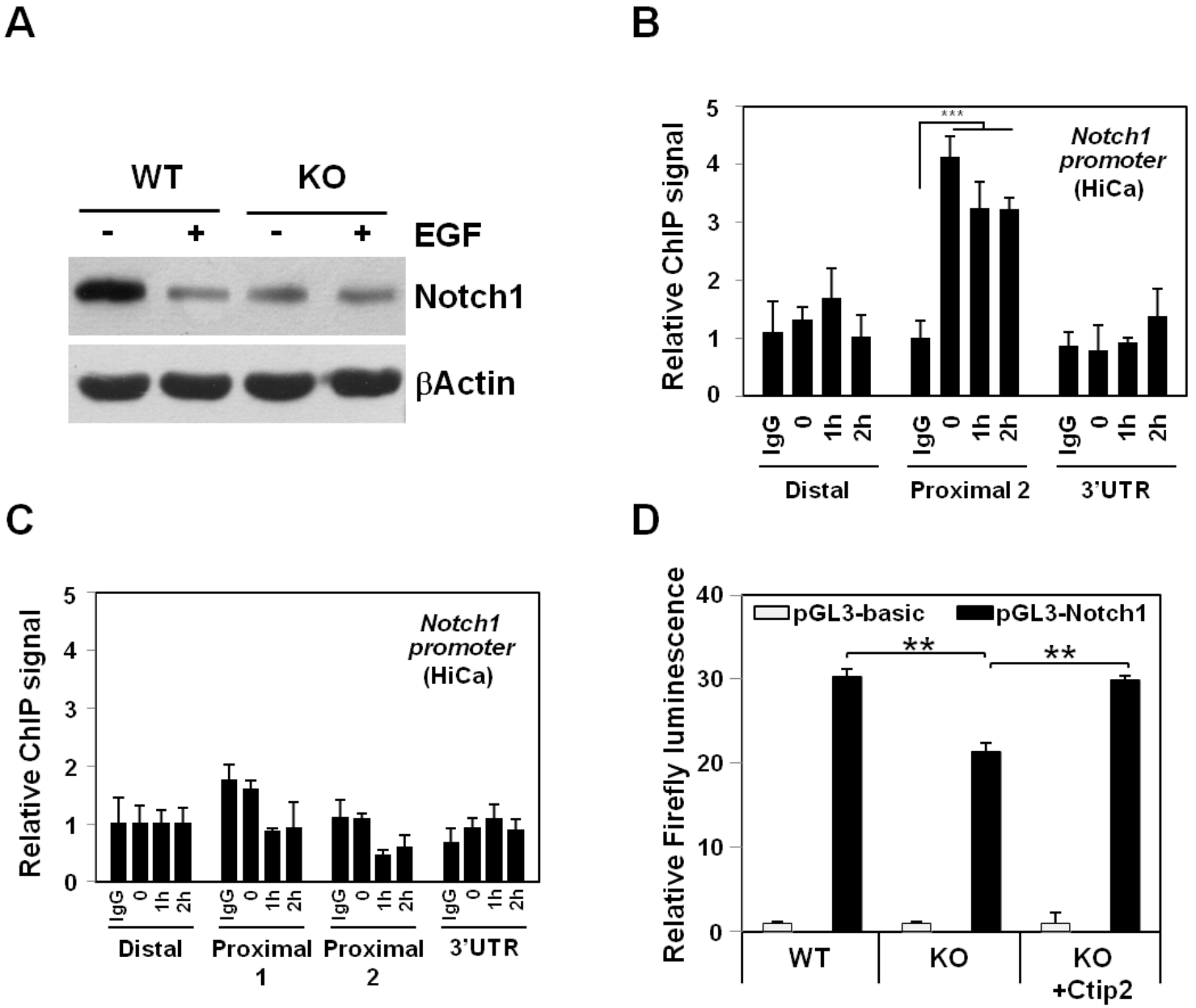

Fig. S5. Coordinate regulation of Notch1 by Ctip2. (A) Negative regulation of Notch1 expression by EGF signaling. Immunoblotting analyses of cell extracts from starved WT and KO keratinocytes treated with or without EGF using anti-Notch1 and anti-βactin antibodies as indicated. βactin was used as a loading control. **(B)** ChIP assays were performed in starved keratinocytes treated with 0.2 mM $CaCl_2$ as described in Fig. 6H. In addition, here we show Ctip2 ChIP results using an additional primer pairs for the proximal promoter of Notch1. **(C)** Negative controls for Ctip2 ChIP assays shown in Fig. 6H. ChIP assays were performed as described in Fig. 6H, except that it was performed using E16.5 epidermal keratinocytes from Ctip2 KO pups. **(D)** Ctip2 positively regulates Notch1 promoter in cultured keratinocytes. Wild-type (WT) and Ctip2-null (KO) keratinocytes were co-transfected with a promoterless luciferase construct (pGL3-basic) or luciferase reporter constructs bearing Notch1 promoter with or without an expression vector encoding Ctip2 as indicated. Relative firefly luminescence were displayed as described in Fig. S4A. Statistical significance was determined by Student's un-paired *t*-test using the GraphPad Prism software (**P<0.01). All experiments were performed in triplicates.

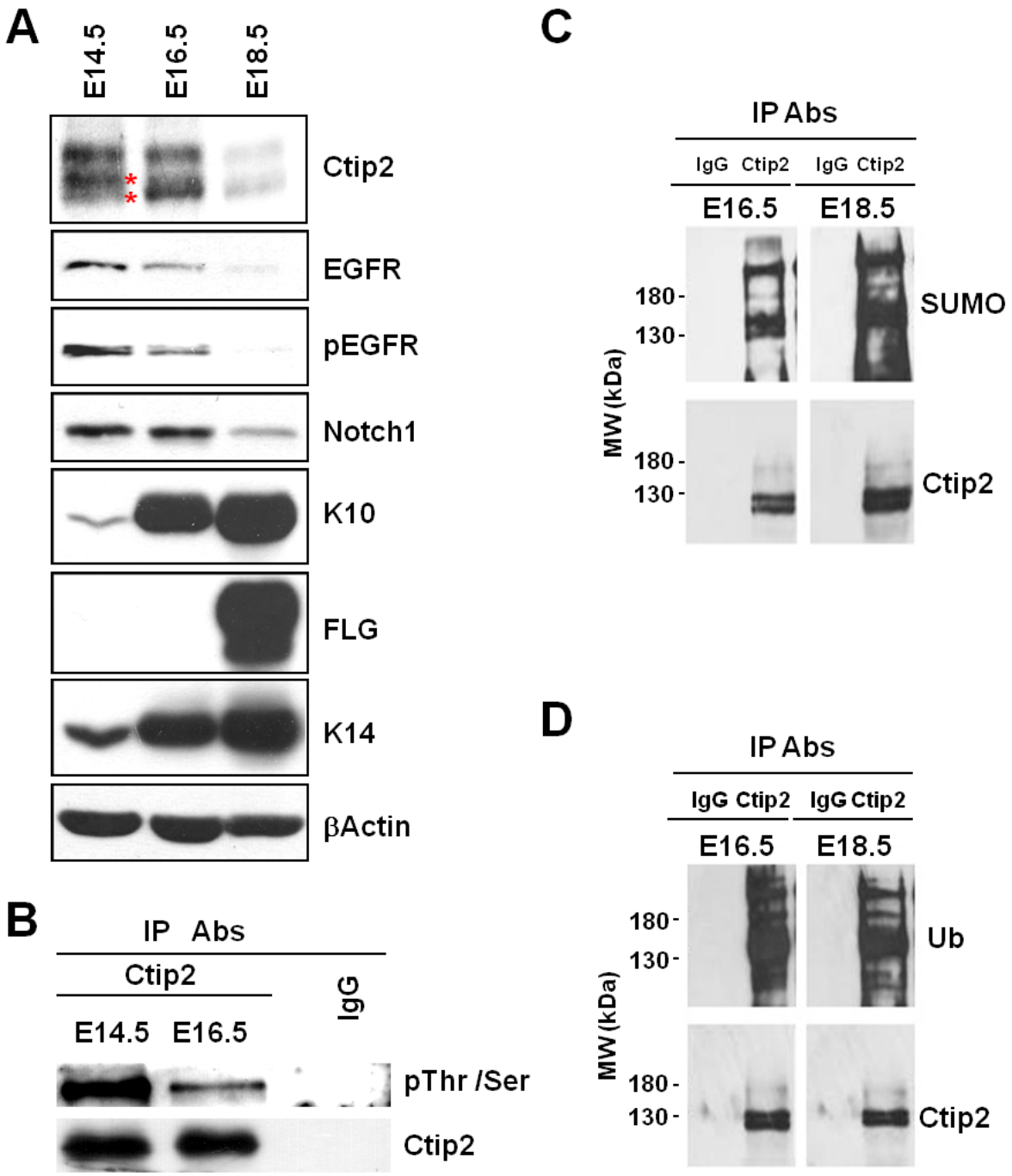

Fig. S6. Endogenous Ctip2 is regulated by post-translational modifications in developing epidermal keratinocytes. **(A)** Coordinated expression of Ctip2, EGFR, and Notch1 in developing mouse skin. Whole cell extracts prepared from dorsal skin biopsies collected at indicated embryonic stages of development were subjected to immunoblotting analyses using anti-Ctip2, -EGFR, -phospho-EGFR, -Notch1, -K10, -FLG, and -K14 antibodies. β-actin blot was shown as loading control. EGF-induced phosphorylation of Ctip2 leads to a small reduction in the electrophoretic mobility of Ctip2 on SDS-PAGE. Using E14.5 whole cell extract, we noticed an electrophoretic mobility shift of the smaller splice variant Ctip2 (indicated by asterisks; the lower asterisk indicates unmodified Ctip2 and the upper asterisk indicates the slower migrating Ctip2). These results suggest that Ctip2 is phosphorylated at E14.5, a stage at which EGFR signaling is highly activated (as indicated by pEGFR). The level of Ctip2 mobility shift decreased concomitantly with the decrease in the levels of EGFR phosphoryation. **(B)** Ctip2 is phosphorylated in the developing skin. E14.5 and E16.5 embryonic skin was harvested. IP experiments were carried out using anti-Ctip2 antibody or IgG as control and the immunoprecipitates were probed with an anti-phospho-threonine antibody to detect phosphorylated Ctip2 (the upper blot). The blot was then stripped and reprobed with the anti-Ctip2 antibody (the lower blot). **(C-D)** Ctip2 is SUMOylated (C) and ubiquitinated (D) in epidermal keratinocytes at E16.5 and E18.5. Lysates from epidermal keratinocytes at E16.5 or E18.5 were subjected to IP analyses using either Ctip2 antibody or IgG as control. Immunoprecipitates were probed with an anti-SUMO1 antibody to detect SUMOylated Ctip2 (**C**, the upper blot) or anti-ubiquitin antibody to detect ubiquitinated Ctip2 (**D**, the upper blot). The blots were then stripped and reprobed with the anti-Ctip2 antibody (the lower blots). All experiments were performed in triplicates.

Table S1. Antibodies

Antibody	Species	Source/Company	Dilution for IB	Dilution for IF
anti-Ctip2	rat	Abcam (Cambridge, MA)	1:2000	1:300
anti-PCNA	mouse		1:3000	
anti-SUMO1	rabbit		1:2000	
anti-Ubiquitin	mouse	Enzo life science (Plymouth Meeting, PA)	1:2000	
anti-E-Cadherin	rabbit	Cell Signaling (Danvers, MA)	1:1000	1:200
anti-phospho-EGFR	rabbit		1:1000	1:200
anti-EGFR	rabbit		1:1000	
anti-Notch1	rabbit		1:1000	1:200
anti-phospho-ERK1/2	rabbit		1:2000	
anti-ERK1/2	rabbit		1:2000	
anti-phospho- Elk-1 (Ser 383)	rabbit		1:1000	
anti-phospho-threonine/ serine	mouse		1:2000	
anti-K14	rabbit	Covance (Princeton, NJ)	1:5000	1:1000
anti-K10	rabbit		1:5000	1:1000
anti-Filaggrin (FLG)	rabbit		1:1000	1:500
anti-Involucrin (INV)	rabbit		1:1000	1:1000
anti-loricrin (LOR)	rabbit		1:1000	1:1000
anti-bactin	rabbit	Bethyl Laboratories (Montgomery, TX)	1:2000	
anti-Ki67	mouse	NovaCastra (Bannockburn, IL)	1:500	1:100
TRITC-phalloidin		Sigma (St. Louis, MO)	1:1000	
anti-p21	mouse	Santa Cruz (Santa Cruz, CA)	1:200	
anti-Keratin 8 (K8)	rat	Developmental Studies Hybridoma Bank (DSHB)	1:1000	

Abbreviation used: immunoblotting (IB); immunofluorescence (IF)

Table S2. List of primers used for RT-qPCR

Gene	Strand	Primer sequence
Calb1	Forward	AGCGGCTTCATCGAAACCG
	Reverse	TCAGCATGAGGTCTGTGTACT
CasR	Forward	AGCAGGTGACCTTCGATGAGT
	Reverse	ACTTCCTTGAACACAATGGAGC
Cdc42	Forward	CCCATCGGAATATGTACCAACTG
	Reverse	CCAAGAGTGTATGGCTCTCCAC
c-Fos	Forward	CGGGTTTCAACGCCGACTA
	Reverse	TTGGCACTAGAGACGGACAGA
Csk	Forward	TTCCCTTCTGCAAAGGAGATGT
	Reverse	ACCAGGGCATAAGGCTGAGT
EGFR	Forward	GCCATCTGGGCCAAAGATACC
	Reverse	GTCTTCGCATGAATAGGCCAAT
FAK	Forward	CCATGCCCTCGAAAAGCTATG
	Reverse	TGACGCATTGTTAAGGCTTCT
FLG	Forward	GGACAACTACAGGCAGTCTTGAAGA
	Reverse	CATTTGCATGAAGACTTCAGCG
HPRT	Forward	GTTAAGCAGTACAGCCCCAAA
	Reverse	AGGGCATATCCAACAACAAACTT
K1	Forward	TGGGAGATTTTCAGGAGGAGG
	Reverse	GCCACACTCTTGGAGATGCTC
K8	Forward	GATGAGATCCAACAGCGTACAG
	Reverse	CATGTATGCTTCGTCCACATCC
K10	Forward	GTCCACTGGTGATGTGAATGT
	Reverse	CCAGACCCTGAACAGTACGTC
ILK	Forward	TGATGAATCGTGGGGATGATACC
	Reverse	GCATTGGTGTCAGCCTTGTATT
Itga3	Forward	CCTCTTCGGCTACTCGGTC
	Reverse	CCGGTTGGTATAGTCATCACCC
Itga6	Forward	TGCAGAGGGCGAACAGAAC
	Reverse	GCACACGTCACCACTTTGC
Itgb1	Forward	TGTTGGTCAGCAACGCATATC
	Reverse	CACCAGCAGTCGTGTTACATT
Itgb4	Forward	TGGCTACTACACTGTCACGG
	Reverse	CAGGGACATCAATGGCCTCC
MKP3	Forward	ATAGATACGCTCAGACCCGTG
	Reverse	ATCAGCAGAAGCCGTTCGTT
Notch1	Forward	TCAATGCCGTGGATGACCTA
	Reverse	CCTTGTTGGCTCCGTTCTTC
PppR2b	Forward	GCTCTGGTCCCGAGATTTTCA

	Reverse	AGATGATGGCGAACAGGAGAA
Pppr2C	Forward	CTGCGTGCTTACATCAGGAA
	Reverse	TCAGTGGTAAGGCAAATCCA
Rac1	Forward	GAGACGGAGCTGTTGGTAAAA
	Reverse	ATAGGCCCAGATTCACTGGTT
RohA	Forward	AGCTTGTGGTAAGACATGCTTG
	Reverse	GTGTCCCATAAAGCCAACTCTAC
SMAD2	Forward	ATGTCGTCCATCTTGCCATTC
	Reverse	AACCGTCCTGTTTTCTTTAGCTT
SMAD3	Forward	CCCCCACTGGATGACTACAG
	Reverse	TCCATCTTCACTCAGGTAGCC
SMAD4	Forward	ACACCAACAAGTAACGATGCC
	Reverse	GCAAAGGTTTCACTTTCCCCA
FAK	Forward	CCATGCCCTCGAAAAGCTATG
	Reverse	TGACGCATTGTTAAGGCTTCT
Snail1	Forward	CACACGCTGCCTTGTGTCT
	Reverse	GGTCAGCAAAAGCACGGTT
Snail2	Forward	GGCTGCTTCAAGGACACATT
	Reverse	TTGGAGCAGTTTTTGCACTG
TGFα	Forward	CACTCTGGGTACGTGGGTG
	Reverse	CACAGGTGATAATGAGGACAGC
TGFβ1	Forward	CCGCAACAACGCCATCTATG
	Reverse	CTCTGCACGGGACAGCAAT
TGFβ2	Forward	CTTCGACGTGACAGACGCT
	Reverse	GCAGGGGCAGTGTAAACTTATT

Table S3. List of primers used for ChIP

Gene	Promoter region	Amplified region	Strand	Sequence
EGFR	Distal	-2553 to -2705	Forward	tgacttgtgccactgaccat
			Reverse	ggtgctcttacccactgagc
	Proximal-1	-203 to -399	Forward	tctccaacctgtgctctgtg
			Reverse	gggtggcctttggagttaat
	Proximal-2	-54 to -172	Forward	ccccagagccttgtctagtg
			Reverse	ggagcgaagaggaggagaat
	3'UTR	1743 to 1847	Forward	tctggaagtctcccctgcta
			Reverse	cactgcaggctctgttttga
Notch1	Distal	-4484 to -4623	Forward	gggtgggtaaatgtcctcaa
			Reverse	ctgtgtccacacccttcctt
	Proximal-1	-8 to -108	Forward	gttcctagatgcccattcca
			Reverse	atggttcgcaagacaaggag
	Proximal-2	+626 to +789	Forward	tgcgcctctacttttcgatt
			Reverse	cccgctctaagtaagcaacg
	3'UTR	4578 to 4728	Forward	gggaaacaagccaataagca
			Reverse	ttggaggggaaaggagttct
K8	Distal	-2180 to -2270	Forward	aggggagaaagggagagttg
			Reverse	ctgtgagagaaagcggaagg
	Proximal-1	-1489 to -1299	Forward	atgtgcccatagagcagtcc
			Reverse	cacttcagggtgtgatgtgg
	Proximal-2	-914 to -753	Forward	aaagcccctgtgcctctat
			Reverse	aacgcagctcttctctctgg
	3'UTR	483 to 642	Forward	gtgttgggtctgccatttct
			Reverse	cctccacacacacccttttct

Note: Amplified region for distal and proximal promoter is relative to the transcription start site of indicated gene and amplified region for 3'UTR is relative to the transcription stop site of indicated gene.