

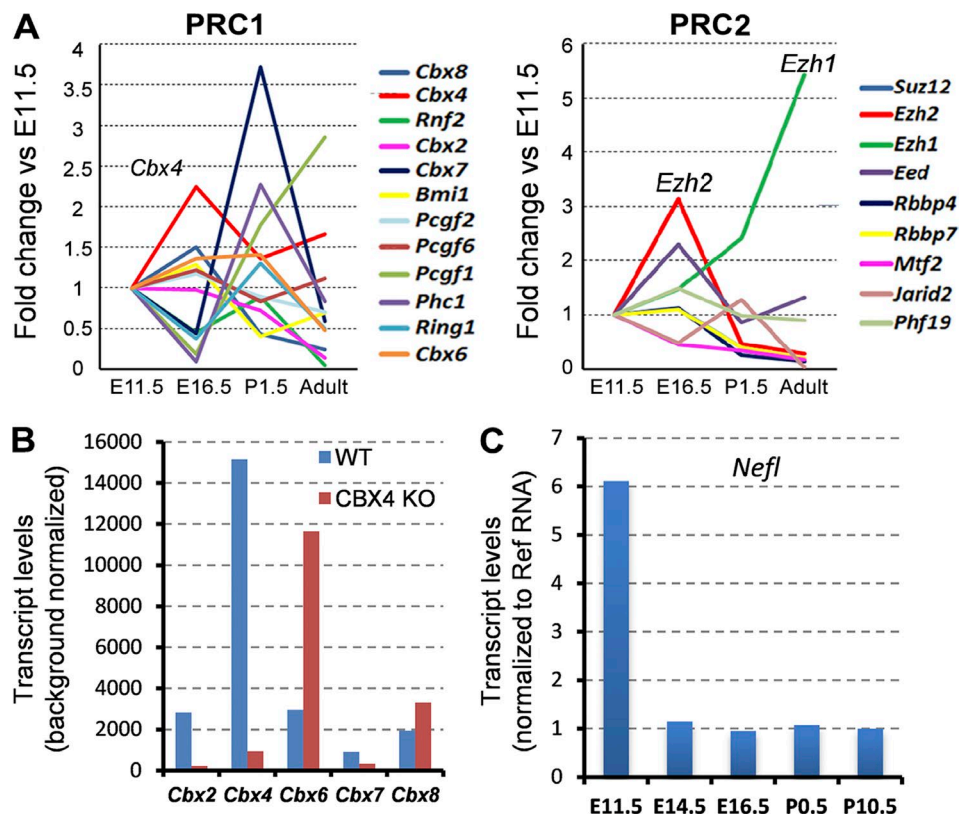
Mardaryev et al., <http://www.jcb.org/cgi/content/full/jcb.201506065/DC1>

Figure S1. **Expression of the PRC1/PRC2 genes and *Nefl* gene in mouse epidermis during development.** (A) Expression of the distinct PRC1 and PRC2 genes in the laser-captured mouse epidermis during distinct stages of development (Agilent microarray values normalized to the reference RNA levels are shown relative to the corresponding expression levels in E11.5 epidermis). The data shown are from a single experiment performed in duplicate. (B) Expression of different *Cbx* genes in the epidermis of E16.5 WT and *Cbx4*KO mice (Agilent microarray, background-normalized data). The data shown are from a single experiment performed in duplicate (mean \pm SD). $n = 2$; two-way *t* test. (C) Expression of the *Nefl* transcripts in WT mouse epidermis at distinct stages of development (Agilent microarray values normalized to the reference RNA levels). The data shown are from a single experiment performed in duplicate (mean \pm SD; $n = 2$; one-way ANOVA).

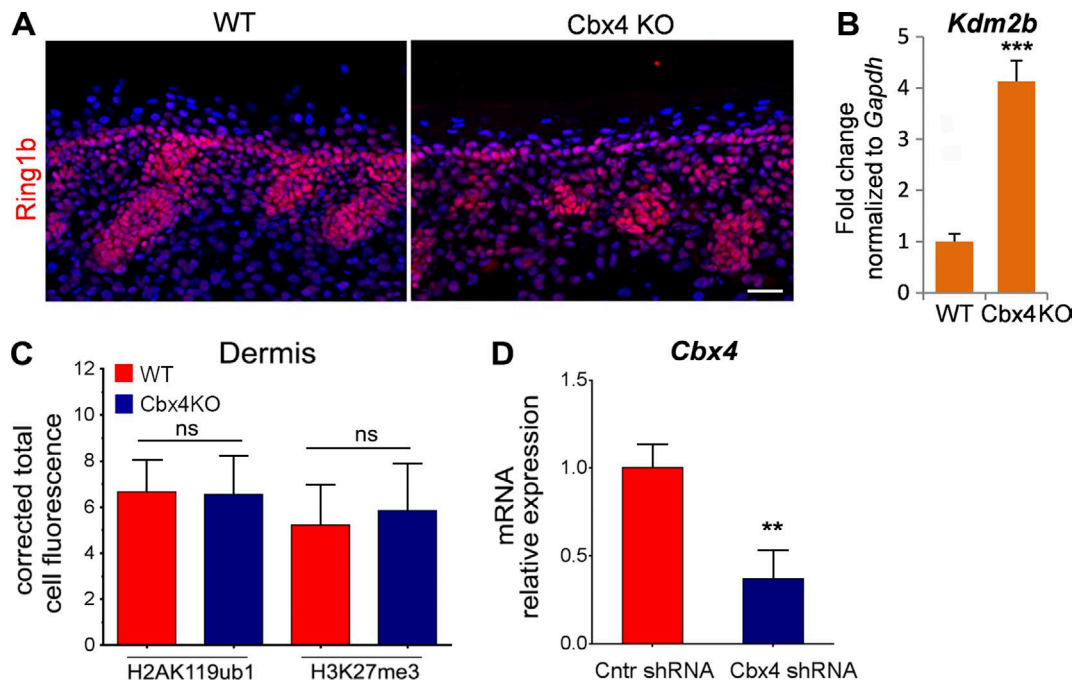


Figure S2. **Characterization of the changes in gene expression in the epidermis of *Cbx4*KO mice and in primary KCs after *Cbx4* knockdown.** (A) Immunofluorescence detection of Ring1b shows similar patterns of expression in the epidermis of E16.5 WT and *Cbx4*KO mice. Bar, 25 μ m. (B) *Kdm2b* transcript levels are significantly up-regulated in the E16.5 epidermis of *Cbx4*KO mice compared with WT controls (mean \pm SD; $n = 3$). (C) Correlated total cell fluorescence level of H2AK119ub1 and H3K27me3 show a lack of differences in their expression between the dermal cells of E16.5 WT and *Cbx4*KO mice. (D) Down-regulation of the *Cbx4* transcripts in the primary mouse KCs after treatment with *Cbx4* shRNA (mean \pm SD; $n = 3$). **, $P < 0.01$; ***, $P < 0.001$. ns, not significant.

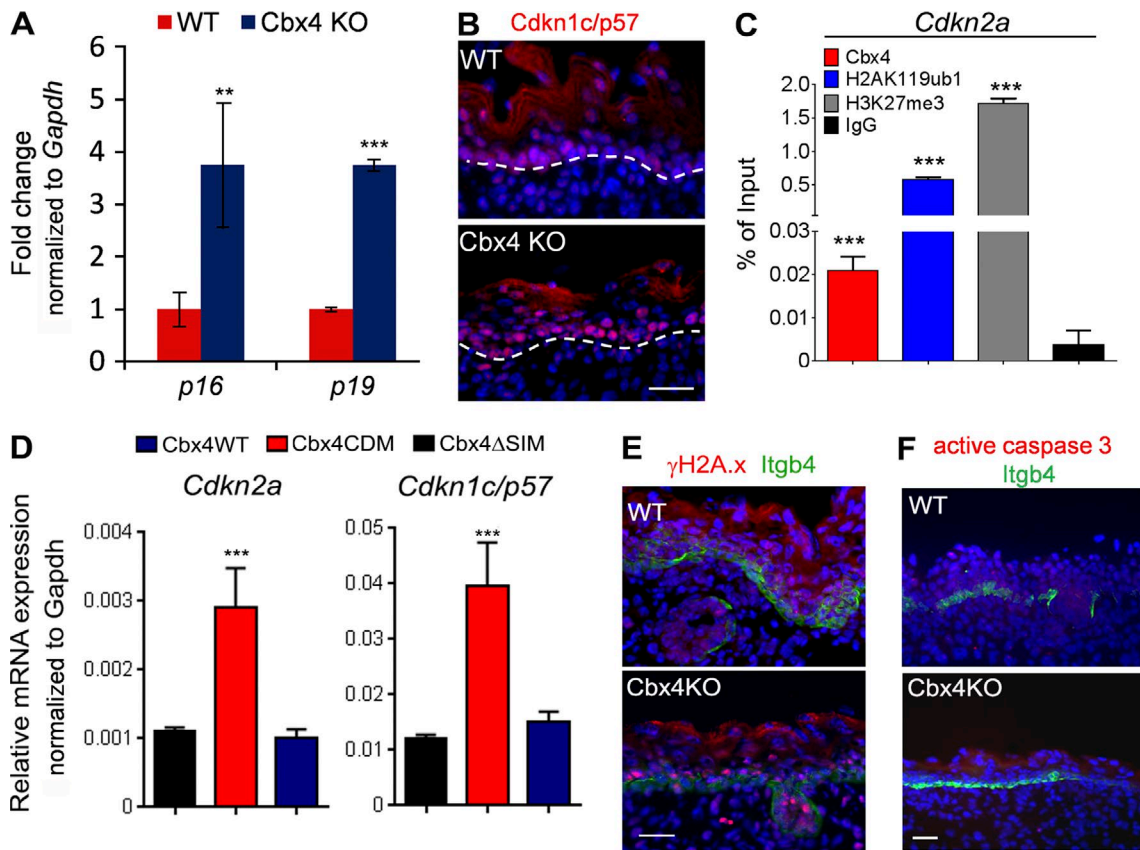


Figure S3. **Analyses of the senescence and apoptotic markers in WT and *Cbx4*-deficient skin.** (A) p16 and p19 transcript levels are significantly up-regulated in the epidermis of E16.5 *Cbx4*KO mice compared with WT controls (mean \pm SD; $n = 3$). (B) Increased *Cdkn1c/p57* protein expression in the epidermis of E16.5 *Cbx4*KO mice. Dashed lines separate epidermis and dermis. (C) ChIP-qPCR analyses show an enrichment for Cbx4, H2AK119ub1, and H3K27me3 in the promoter region of the *Cdkn2a* gene in primary KCs (mean \pm SD; $n = 3$). (D) Increase of the levels of the *Cdkn2a* and *Cdkn1c* transcripts in primary mouse KCs infected with retrovirus expressing a mutant *Cbx4* chromodomain (*Cbx4*CDM; mean \pm SD; $n = 3$). (E) Appearance of γ -H2AX-positive cells in the epidermis of E16.5 *Cbx4*KO mice. Integrin- β 4 (*Itgb4*) expression outlines the basement membrane of the epidermis. DAPI counterstain (blue) shows the nuclei. (F) A lack of caspase 3-positive cells in the epidermis of E16.5 WT and *Cbx4*KO mice. Integrin- β 4 expression outlines the basement membrane of the epidermis. DAPI counterstain (blue) shows the nuclei. Bars, 50 μ m. **, $P < 0.01$; ***, $P < 0.001$.

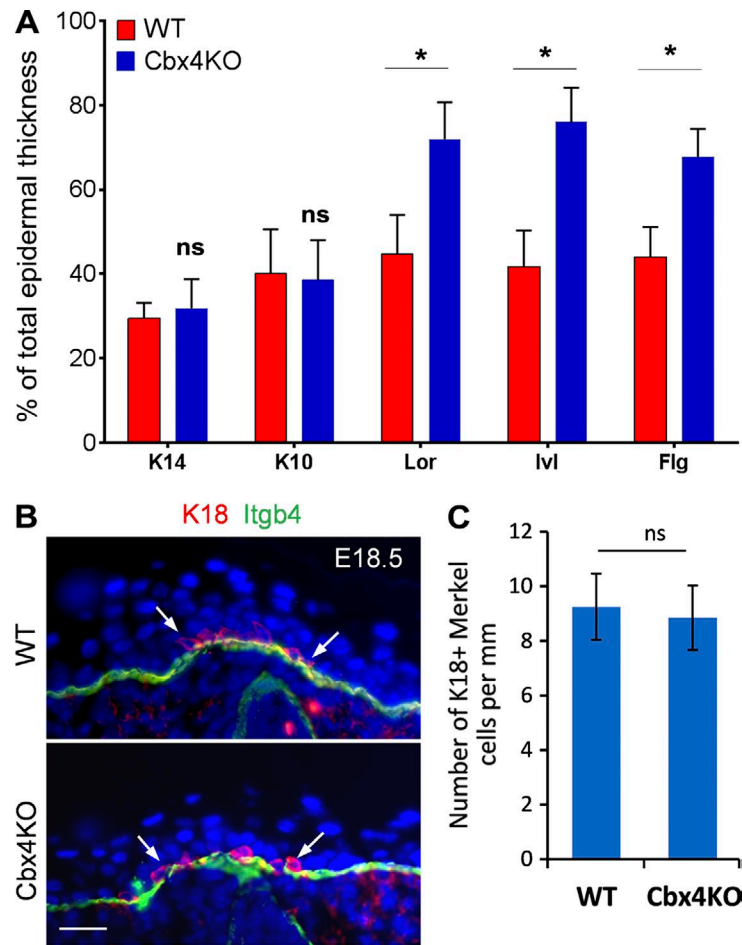


Figure S4. **The analyses of epidermal and Merkel cell differentiation in Cbx4KO and WT mice.** (A) Quantification of the ratios between different cellular layers expressing distinct markers of epidermal differentiation shows a significant increase of the cellular layers expressing the markers of terminal KC differentiation (Loricrin, Involucrin, and Filaggrin) in the epidermis of E16.5 Cbx4KO mice versus WT controls ($P < 0.01$). (B and C) Immunofluorescence analyses of the Merkel cells expressing K18 (B, arrows) show a lack of differences between the epidermis of E16.5 Cbx4KO and WT mice. Integrin- β 4 (Itgb4) expression outlines the basement membrane of the epidermis (C). DAPI counterstain (blue) shows the nuclei. Bar, 50 μ m. *, $P < 0.05$. ns, not significant.

Table S1. Expression of PRC1 and PRC2 genes in epidermal progenitor cells at different stages of epidermal development

Gene	E11.5		E16.5		P1.5		P56	
	Absolute values	Normalized values	Absolute values	Normalized values	Absolute values	Normalized values	Absolute values	Normalized values
PRC1								
<i>Bmi1</i>	4,939	0.78	890	1.00	1,959	0.32	164.19	0.54
<i>Cbx2</i>	10,576	2.69	2,616	2.64	7,870	1.96	1,062.61	0.40
<i>Cbx4</i>	5,757	0.44	5,378	0.99	8,019	0.60	7,709.26	0.74
<i>Cbx6</i>	26,436	1.18	4,686	1.61	39,345	1.67	196.33	0.57
<i>Cbx7</i>	2,643	1.00	28	0.44	12,494	3.73	369.93	0.60
<i>Cbx8</i>	3,995	2.38	2,623	3.58	1,548	1.03	21,910.41	0.59
<i>Pcgf1</i>	3,271	1.27	114	0.24	4,264	2.26	7,096.51	3.64
<i>Pcgf2</i>	387	1.02	22	1.21	384	0.92	230.91	0.72
<i>Pcgf6</i>	1,816	0.58	354	0.71	1,662	0.49	1,946.37	0.65
<i>Phc1</i>	29,160	1.75	135	0.17	68,436	3.99	10,662.08	1.47
<i>Phf19</i>	1,053	0.56	196	0.83	1,198	0.55	788.60	0.51
<i>Ring1</i>	1,179	1.16	69	0.44	1,831	1.51	728.27	0.59
<i>Rnf2</i>	1,006	0.53	28	0.24	945	0.48	181.02	0.03
<i>Rybp</i>	222	0.53	134	1.27	208	0.50	221.50	0.56
PRC2								
<i>Eed</i>	2,095	0.51	91	1.17	1,960	0.44	3,339	0.67
<i>Ezh1</i>	749	1.45	122	2.13	1,672	3.49	3,882	7.89
<i>Ezh2</i>	2,341	0.43	484	1.36	1,098	0.20	671	0.12
<i>Jarid2</i>	8,854	4.31	4,561	2.08	11,294	5.55	304	0.18
<i>Mtf2</i>	6,840	4.10	3,727	1.34	2,968	1.70	1,582	0.71
<i>Mtf2</i>	17,216	3.74	885	1.72	6,605	1.26	2,960	0.67
<i>Rbbp4</i>	28,626	2.36	10,711	2.67	6,897	0.62	4,209	0.34
<i>Rbbp7</i>	36,889	0.59	5,890	0.65	15,775	0.24	9,247	0.12
<i>Suz12</i>	14,478	0.82	2,603	0.92	4,917	0.33	1,909	0.12

Agilent Technologies whole genome microarray values: absolute expression levels (a.u.) and normalized to reference RNA.

Table S2. **Cbx4 target genes up-regulated in p63KO epidermal progenitor cells**

Gene name	p63 versus WT
<i>O610040J01Rik</i>	2.12
<i>1700056E22Rik</i>	3.42
<i>Acot12</i>	3.04
<i>Atp1a2</i>	2.13
<i>BC051665</i>	7.15
<i>C8b</i>	4.38
<i>Cbr4</i>	2.4
<i>Cfi</i>	2.01
<i>Chmp2b</i>	7.78
<i>Ckmt2</i>	2.33
<i>Clybl</i>	5.01
<i>Cpn2</i>	6.31
<i>Ctdp1</i>	16.58
<i>Cyp4f15</i>	2.21
<i>Daglb</i>	12.98
<i>Dmrt2</i>	95.42
<i>Dmrt3</i>	5.63
<i>Dpysl3</i>	3.11
<i>Dtx1</i>	2.26
<i>Dusp26</i>	4.37
<i>Ehd3</i>	3.13
<i>Elovl5</i>	2.47
<i>Eml4</i>	5.7
<i>Enpep</i>	50.8
<i>Fer1l4</i>	2.62
<i>Fez1</i>	3.97
<i>Fndc3c1</i>	16.5
<i>Foxp2</i>	2.16
<i>Grem2</i>	28.43
<i>Hpd</i>	3.47
<i>Kcnq1</i>	2.99
<i>L2hgdh</i>	2.52
<i>Lect2</i>	8.96
<i>Lpl</i>	2
<i>Lrrc17</i>	3.18
<i>Lypd1</i>	3.03
<i>Mrps31</i>	2.02
<i>Nefl</i>	23.26
<i>Nsg1</i>	3.67
<i>Pah</i>	114.77
<i>Parp16</i>	4.35
<i>Pcmd2</i>	3.3
<i>Pdzrn3</i>	2.35
<i>Pon1</i>	20.73
<i>Psmc6</i>	2.34
<i>Rasgef1b</i>	3.04
<i>Rpap3</i>	3.04
<i>Rwdd2a</i>	5.57
<i>Rxrg</i>	2.97
<i>Sfrp2</i>	3.34
<i>Shisa2</i>	6.53
<i>Slc37a3</i>	7.02
<i>Slc6a12</i>	3.06
<i>Slc9a2</i>	56.62
<i>Slco2b1</i>	3.16
<i>Sord</i>	4.33
<i>Sox7</i>	21.69
<i>Stc2</i>	25.25
<i>Stmn4</i>	4.39
<i>Sulf1</i>	5.77
<i>Sulf2</i>	2.56

Table S2. **Cbx4 target genes up-regulated in p63KO epidermal progenitor cells (Continued)**

Gene name	p63 versus WT
<i>Tdrd7</i>	2.82
<i>Tmem132c</i>	8.1
<i>Traf3</i>	3.54
<i>Tshz3</i>	88.53
<i>Tspan12</i>	10.11
<i>Unc5cl</i>	2.71
<i>Upb1</i>	2.16
<i>Utp14b</i>	4.64
<i>Zbp1</i>	135.35
<i>Zfp518b</i>	5.81
<i>Zfp521</i>	6.49
<i>O61004OJ01Rik</i>	10.01
<i>1700056E22Rik</i>	5.85

Table S3. **Primers used for qRT-PCR analyses**

Gene	Oligos 5' - to -3'
qRT-PCR primers	
<i>Cbx4</i>	AGTGGAGTATCTGGTAAATGGA TCCTGCCTTCCCTGTTCTG
<i>Cdkn2a/p16</i>	ATGGGTCGCAGGTTCTTGGT ATCATCACCTGGTCCAGGATTCC
<i>Cdkn2d/p19</i>	CCTTGCAGGTCATGATGTTTGG CCAGGGCATTGACATCAGCA
<i>En2</i>	CTTCTTCAGGTCCAGGT CAAATCTTGATCTGAGACTCGT
<i>Flg</i>	GAAGGAACTTCTGAAGGACAAC TCCATCAGTCCACCATGCCTC
<i>Gapdh</i>	GTGTTCTACCCCCAATGTG AGGAGACAACCTGGTCTCA
<i>Ivl</i>	GCAGGAGAAGTAGATAGAG TTAAGGAAGTGGATGG
<i>Lhx4</i>	CCGATGCAACAGATTCCC GAAGCATCTGCAGCCAG
<i>Kdm2b</i>	GATGCTGAGCGGTATCATCCG GAGACAGCGATCCATGAGCAG
<i>Mobp</i>	CCAGGCTCTCAAGAACCAG GGTCCACGATCTCAGCTT
<i>Nefl</i>	CGCCATGCAGGACACAATCA GAGTAGCCGCTGGTTATGCT
<i>Neurog3</i>	TCGTCTTACTGCCCGCTAC CTAGGGCTTCCGGTTCACA
<i>Olig2</i>	TCCCAGAACCCGATGATCTT CGTGGACGAGGACACAGTC
<i>Zeb2</i>	ATTGCACATCAGACTTTGAGGAA ATAATGGCCGTGTCGCTTCG
Cloning primers	
<i>Cbx4</i> enhancer	AATCGATAAGGATCCTTTGAGAACAGAACAAGGGT AGGGCATCGGTCGACGGATTTCCAGACAGCCAAGG
ChIP primers	
<i>Cbx4</i>	TGTGTCAAAGAGACTAAGGACAGC AGCTCCAGTGCTCAGTGGTC
<i>Lor</i>	ACCAGTTACCACTCTCCCA GTCTGGTCTCTCCAGTTGGC
<i>Nefl</i>	GTCAGAGTCCCGGCTATAA AGCCGAACGAACTCATGGTG
<i>Cdkn2a</i>	TCCCTCCGGTTAACTTTCGG GCCTCGCCGATCTTCTATT

Table S4. List of antibodies used in this study

Antigen	Application	Host	Dilution	Catalog number	Supplier
Caspase 3 active	IF	Rabbit	1:200	ab13847	Abcam
Cbx4	ChIP	Rabbit	5 µg	A302-355A	Bethyl Laboratories, Inc.
Cbx4	IF	Rabbit	1:100		This study/polyclonal antibody against amino acids 363–551
CD104 (Itgb4)	IF	Rat	1:100	553745	BD
Cdkn1c	IF	Rabbit	1:100	ab4058	Abcam
Filaggrin	IF	Rabbit	1:100	sc-30230	Santa Cruz Biotechnology, Inc.
gH2A.x	IF	Rabbit	1:100	39117	Active Motif
H2AK119ub1	IF/ChIP	Rabbit	1:200	8240s	Cell Signaling Technology
H3K27me3	IF/ChIP	Rabbit	1:100; 5 µg	39157	Active Motif
Involucrin	IF	Rabbit	1:100	ab53112	Abcam
Ki67	IF	Rabbit	1:100	ab15580	Abcam
Krt10	IF	Rabbit	1:100	ab76318	Abcam
Krt14	IF	Rabbit	1:100	ab7800	Abcam
Krt18	IF	Rabbit	1:100	sc-31700	Santa Cruz Biotechnology, Inc.
Loricrin	IF	Rabbit	1:1,000	PRB-145P	Covance
Nefl	IF	Rabbit	0.111111	NB300-131	Novus Biologicals
Ring1b	IF	Rabbit	1:100	5694P	Cell Signaling Technology

IF, immunofluorescence.

Table S5 is supplied as a PDF and it shows Cbx4 target genes in epidermal KCs.