

Supplementary Figure Legends

Figure 1. (A) Expression of p63, Smad7, Bmp7 and Bmp6 mRNA was determined by in situ hybridization in newborn mouse skin at P1. Frozen sections were hybridized with digoxigenin-labeled antisense probes. Corresponding sense cRNAs were used as control. The dashed lines indicate the dermal–epidermal junction. Scale bar 60µm. (B) In situ hybridization of Bmp7 mRNA was performed on frozen sections of p63 null (KO) and wild-type (WT) embryos at E14.5. Scale bar 50µM. (C) The predicted p63-binding hemi-sites located at +2.4Kb from the Bmp7 TSS are indicated with their nucleotide sequence and phylogenetic conservation in multiple species (Left panel). Bold nucleotides correspond to the core nucleotide sequence required for p63-binding, while underlined nucleotides are matches in the consensus sequence. ChIP followed by real time PCR (right panel) was performed as described in Fig. 3B.

Figure 2. (A) Keratinocytes were transfected with p63, Smad7 and ctr siRNA, and the indicated proteins were detected by immunoblotting. (B) Keratinocytes were transfected with pBABE-Smad7 (Smad7) or with an empty pBABE vector (ctr) and the indicated reporters. Luciferase activity was measured 48 hrs after transfection as described in Figure 1. (C) Krt8 expression was suppressed by two unrelated Smad7 siRNA oligonucleotides (S7 and S7_2) in the absence of p63, confirming Smad7 siRNA specificity. (D) mRNA expression of Lce1a2 and Lce1d was measured by real time RT-PCR in primary keratinocytes transfected with p63, Smad7, p63 and Smad7 (p63/S7), or ctr siRNA 48 hrs after transfection. Cells were either treated with BMP7 for the last 24 hrs or left untreated. Values are normalized for Gapdh expression.

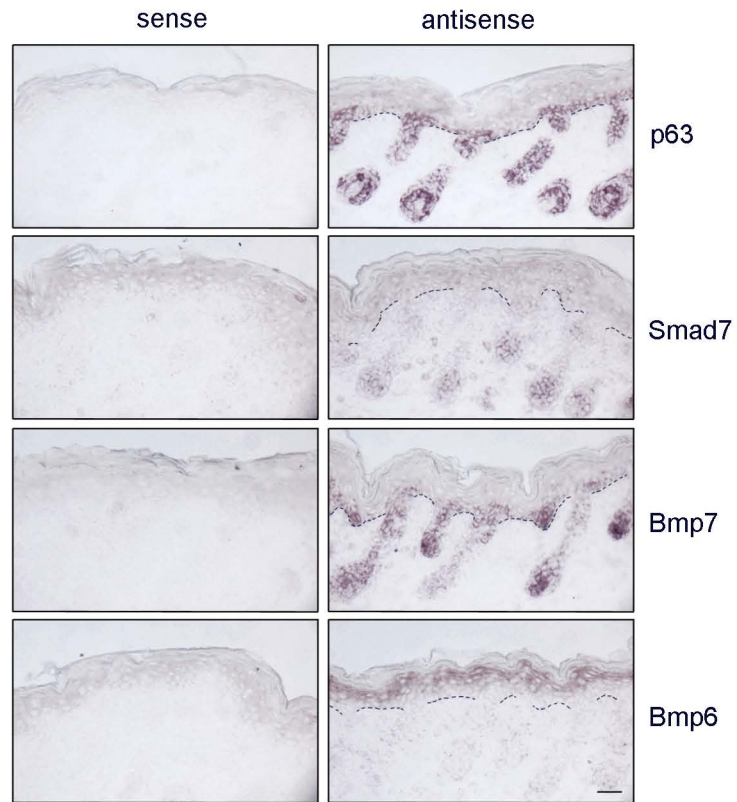
Figure 3. Smad1 (A) and Smad5 (B) expression levels were measured by real time RT-PCR in primary keratinocytes transfected with two unrelated Smad1 (S1_a and S1_b), two unrelated Smad5 (S5_a and S5_b), or ctr siRNA. Values are normalized for β-actin expression. (C) Id1 expression was measured by real time RT-PCR in primary mouse keratinocytes transfected with siRNA for ctr, Smad1 (S1_a), Smad5 (S5_a), or both Smad1 and Smad5.

Supplementary Materials and Methods

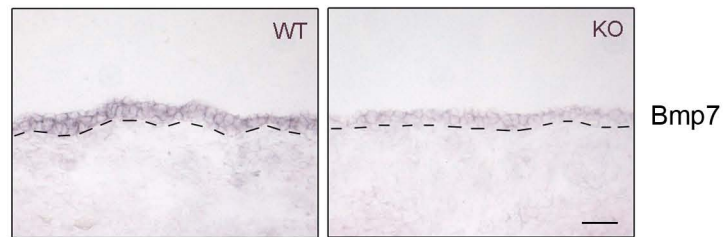
The following primary antibodies were used for immunofluorescence staining: p63 (4A4, Santa Cruz Biotechnology), Krt8 (Troma-1 rat monoclonal antibody developed by Rolf Kemler, obtained from the Developmental Studies Hybridoma Bank at The University of Iowa), Cldn7 (34-9100, Invitrogen), Cldn 3 (34-1700, Invitrogen), Cdh1 (610181, BD Biosciences) for paraffin-embedded tissue, Cdh1 (13-1900, Invitrogen) for frozen-embedded tissue, phospho-Smad1/5/8 (9511, Cell Signaling), Smad1 (sc-7965, Santa Cruz), phospho-Smad2 (3108, Cell Signaling), Smad2 (3103, Cell Signaling). Alexa Fluor® secondary antibodies (Invitrogen) were used for detection. The following primary antibodies were used for immunoblotting analysis in addition to the ones listed above: Smad7 (MAB2029, R&D Systems), polyclonal anti-Bex1 (a gift from Frank Margolis), ERK-1/2 (K-23, Santa Cruz Biotechnology), β-actin (AC-15 Sigma). The following secondary antibodies were used for immunofluorescence staining: Alexa Fluor® 488 goat anti-mouse (Invitrogen), Alexa Fluor® 594 goat anti-rabbit (Invitrogen), Alexa Fluor® 594 goat anti-rat (Invitrogen).

Suppl. Figure 1

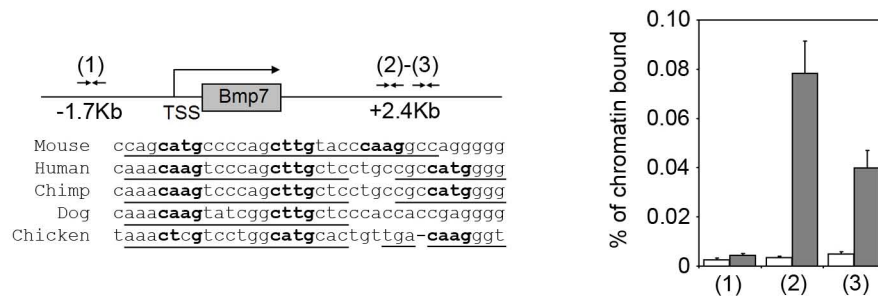
A



B

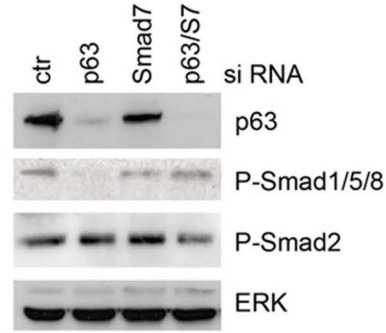


C

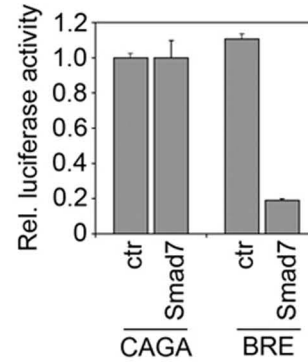


Suppl. Figure 2

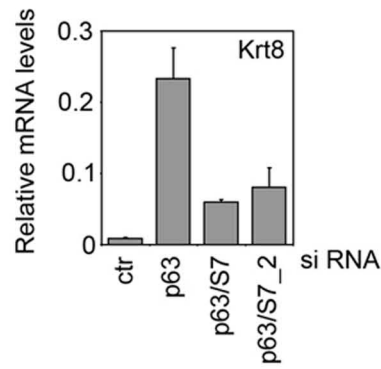
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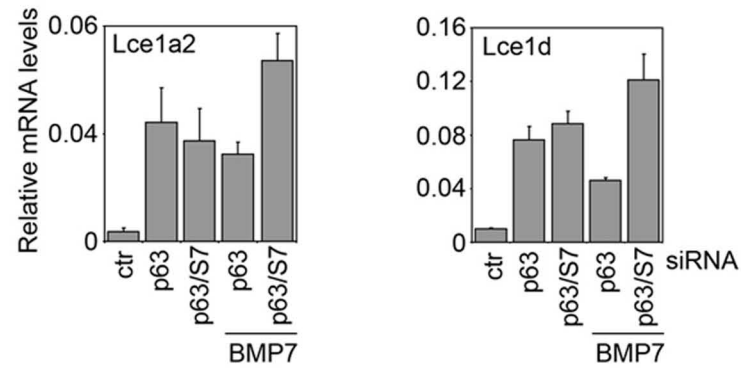
B



C



D



Suppl. Figure 3

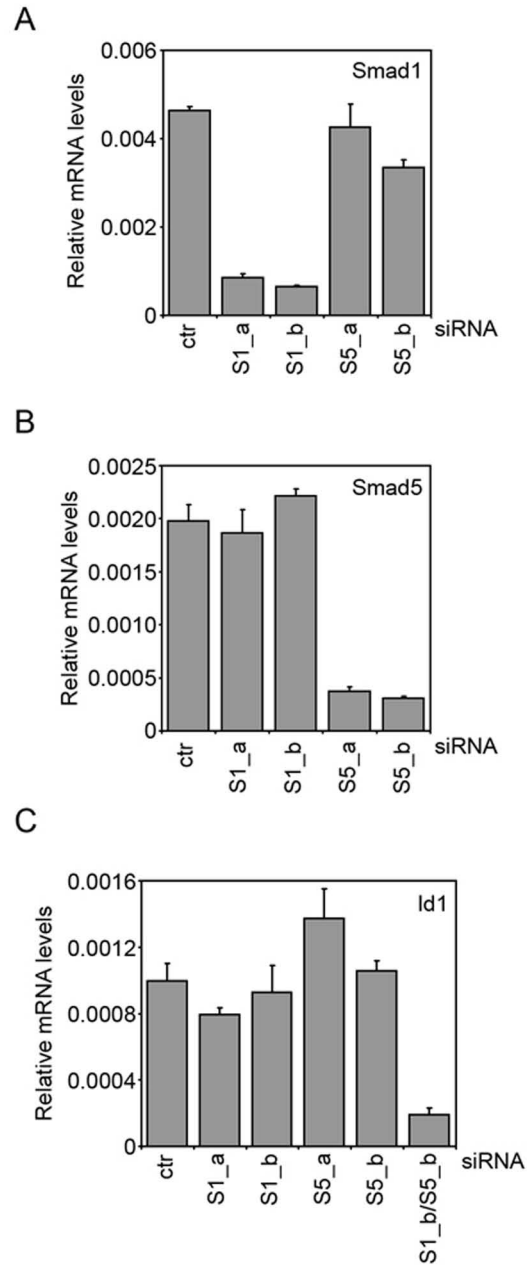


Table S1. Genes induced by p63 knockdown.

Probe Set ID	Gene Symbol	p63KD/ctr	FDR	GNF expression ^a
1451287_s_at	2810003C17Rik	9.6	0.142	U ^b
1436344_at	5830404H04Rik	6.0	0.039	ND ^c
1424838_at	A330049M08Rik	5.8	0.044	trigeminal
1455180_at	AA407270	7.3	0.024	ND
1422925_s_at	Acot3	6.8	0.094	liver, kidney
1427735_a_at	Acta1	5.6	0.090	skeletal muscle, epidermis, snout epidermis, tongue, prostate, bone, brown fat
1421172_at	Adam12	5.5	0.132	U
1450065_at	Adcy7	6.4	0.040	B cells, CD8+ positive cells
1454890_at	Amot	5.0	0.174	placenta, embryo day E6.5
1450325_at	Angpt4	5.4	0.248	snout epidermis
1420991_at	Ankrd1	10	0.234	heart, umbilical cord
1418858_at	Aox3	8.9	0.068	liver, lung, trachea
1455826_a_at	Bace1	7.4	0.155	U
1448595_a_at	Bex1	8.2	0.055	embryo E6.5, E7.5, E8.5
1448752_at	Car2	18.1	0.031	stomach, bone marrow, bone
1419609_at	Ccr1	6.4	0.076	U
1460569_x_at	Cldn3	17.5	0.039	intestin, prostate, blastocyst
1448393_at	Cldn7	7.4	0.145	small intestin, large intestin, blastocysts, uterus
1437689_x_at	Clu	22.0	0.028	bladder, liver, stomach, lymphonode, mammary gland, brain
1419872_at	Csf1r	5.2	0.051	U
1415803_at	Cx3cl1	6.0	0.178	cortex, dorsal striatum, olfactory bulb

1450839_at	D0H4S114	5.3	0.152	cerebellum
1427289_at	Ddhd1	6.4	0.091	testis, ovary, trachea, small intestine, brain
1448619_at	Dhcr7	6.8	0.011	adrenal gland, snout epidermis
1418937_at	Dio2	7.9	0.068	uterus, brown fat
1451270_at	Dusp18	5.6	0.159	U
1417023_a_at	Fabp4	5.0	0.113	brown fat, adipose tissue, ovary
1424647_at	Gabrp	5.7	0.025	trachea
1437618_x_at	Gpr85	6.1	0.198	brain
1436917_s_at	Gpsm1	5.6	0.232	olfactory bulb, heart
1424553_at	Hhatl	5.9	0.181	heart, skeletal muscle
1418352_at	Hsd17b2	12.7	0.023	large intestine, liver, placenta
1427029_at	Htra3	10.1	0.037	epidermis, snout epidermis, digits, placenta, adipose tissue
1417141_at	Igtp	5.4	0.132	CD4+ cells
1435945_a_at	Kcnn4	7.1	0.100	CD4+ cells, CD8+ cells, thyroid
1448169_at	Krt18	17.0	0.058	blastocyst, placenta (stomach, large intestine)
1423691_x_at	Krt8	34.1	0.103	large intestine, blastocyst, stomach, small intestine
1420350_at	Lce1a2	6.7	0.249	digits, epidermis, snout epidermis, tongue
1419409_at	Lce1b	6.7	0.207	digits, epidermis, snout epidermis, tongue
1420332_x_at	Lce1d	5.1	0.210	digits, snout epidermis, tongue
1421316_at	Lce1g	16.2	0.118	epidermis, digits, pancreas
1449959_x_at	Lce1h	6.3	0.148	digits, snout epidermis, tongue
1417275_at	Mal	5.8	0.030	substantia nigra, eye, spinal cord, bladder, stomach, umbilical, cord
1430837_a_at	Mbd1	7.9	0.242	U
1417256_at	Mmp13	5.9	0.096	vomero nasal epithelium, bone
1460238_at	Msln	16.4	0.028	umbilical cord, trachea (pleura)

1416808_at	Nid1	5.5	0.048	adipose tissue, blastocyst
1419665_a_at	Nupr1	5.7	0.012	pancreas, thyroid, salivary gland, vomeronasal olfactory epithelium
1426562_a_at	Olfm1	5.2	0.184	frontal cortex, cerebral cortex, hyppocampus, amygdala, dorsal striatum, bulb olfactory epithelium
1436851_at	Pkn1 /// Ptger1	7.5	0.024	B-cells
1452517_at	Plekhh1	6.3	0.071	placenta, blastocyst
1419700_a_at	Prom1	5.6	0.049	eye, uterus, large intestin, vomero nasal
1420352_at	Prss22	5.9	0.170	ND
1451258_at	Psca	6.3	0.121	bladder, stomach
1436448_a_at	Ptgs1	5.1	0.061	bladder, epidermis, digits
1422324_a_at	Pthlh	5.2	0.020	snout epidermis, vomeronasal epithelium
1451452_a_at	Rgs16	11.2	0.199	mammary gland
1427020_at	Scara3	10.9	0.035	trachea, digits, main olfactory epithelium
1450956_at	Scd3	6.5	0.050	eye, epidermis
1437052_s_at	Slc2a3	20.1	0.024	blastocyst, embryo E6.5, E7.5, E8.5, testis
1450032_at	Slco2a1	10.1	0.070	placenta
1420562_at	Slurp1	6.9	0.173	ND
1436790_a_at	Sox11	8.5	0.129	embryo E9.5, E10.5
1451601_a_at	Spns2	7.1	0.077	U
1420377_at	St8sia2	10.5	0.034	U
1439567_at	Tbx3	9.8	0.132	adrenal gland, prostate, stomach, lung
1417455_at	Tgfb3	13.8	0.034	mammary gland, umbilical cord
1417455_at	Tgfb3	14	0.034	mammary gland, umbilical cord
1450040_at	Timp2	11.0	0.050	U
1419089_at	Timp3	5.0	0.038	adrenal gland, kidney
1419154_at	Tmprss2	5.6	0.025	bladder

1438855_x_at	Tnfaip2	5.5	0.030	bladder, bone marrow, embryo, spleen, liver
1455812_x_at	Vasn	5.6	0.035	ND

^a See Supplementary Material and Methods for GNF expression

^b U: ubiquitous

^c ND: not determined

Table S2. Oligonucleotide primer used in this study.

<u>Oligonucleotide Primers for Real Time RT-PCR</u>		<u>Oligonucleotide Primers for ChIP</u>	
Gapdh	GTATGACTCCACTCACGGCAAA TTCCCATCTCGGCCTTG	Smad7 ctr (-0.2kb)	GCGAAACACAATCGCTTTTTT CGTCACGTGGCCGTCTAGA
Actb	CTAAGGCCAACCGTAAAAAGAT GCCTGGATGGCTACGTACATG	Smad7 (2) (-2.7kb)	ATCTGTTTTTACCCGGGCCT CGTGAGTGGTCTAATCCCCT
p63	CATGAGCTGAGCCGTGAGTTC GGCTGTTCCCTTCTACTCGAA	Smad7 (1) (-3.1kb)	GTGAGGCGAAAGAAGAGCCC GCTCTGACTGGCTTGATGCC
Krt14	TGACGTCTCCACCCACCTG ACCACGAGGAGGAAATGGC	Bmp7 (1) (-1.7kb)	TGCTGTGGTGGGTGATCTGA GTCATGTCTCCAGCTCGCAG
Krt8	TGCTCATGTTCTGCATCCCA GATCACCACTACCGCAAGC	Bmp7 (2) (+2.2kb)	AGGCAAGCGCTGAATTGT TGTGATCTCCAGCAAGGCAGT
Cldn7	ACAGGAGCAAGAGAGCAGGG CTGCCATCTTATCGGCTGG	Bmp7 (3) (+2.5kb)	CCTCGCTGATTGAGAGGCTC TCATGTTGAAAGGACAAAGCCTAG
Tmprss2	CCTACATACAGACTAAATGTGCAA GAAATAACCAACCAACAGCAAAGA		
Bex1	TGACCACCATGATGAGTTTTGC TCCCATGTCATCTTCAGAGAA	<u>siRNA oligonucleotides</u>	
Smad6	GGCTGTCTCCTCCTGACCAGTA CAATGTAGAATCGGACAGATCCAG	Smad7	AGTCAAGAGGCTGTGTTGCTGTGAA
Smad7	GAAGGTGGTGCCACTTTCA AACGAGAGTCAGCACTGCCA	Smad7_2	CCCATCACCTTAGTCGACTCTGTGA
Bmp7	CATCGTCCAGACTGGTTCA AGCAGGGCTTGGGTACTGTG	Smad1_a	CATATTGGGAAAGGAGTCCACCTTT
Bmp2	GGCCGTTTTCCCACTCATCT CCATCACGAAGAAGCCGTG	Smad1_b	CAATCCTATTTTCATCCGTGCTTAA
Bmp4	TGAGGAGTTTCCATCACGAAGAA CACTGGTCCCTGGGATGTTT	Smad5_a	CAGAGATGTTGAGCCTGTCGCTAT
Bmp6	TGTCCAACAAAAATAGGTCAGAGT AAGTCTTGCAAGGAGCATCAGC	Smad5_b	ATTCATAGTAGACAATCGAACACCA
Lce1d	TCGTCTTGCTCCAGAGCACTCACC GGAGGCTGGCACTGCTGTTGG	Bmp7_a	CCTGAAGGCTATGCTGCCTACTACT
Lce1a2	GTTCTGCTGGCAGGACATCT GCCCAAGGATCTTGTACTGC	Bmp7_b	CAACCTAGTGAACATGACAAAGAA
Id1	GAGCAGCAGGTGAACGTCCT TCCTTGAGGCGTGAGTAGCA		
Id2	TGATGCAGGCTGACGATAGTG TCTTGGACCTGCAGATCGC	<u>Cloning of Bmp7 probe for in situ hybridization</u>	
Smad1	ATTGAAAACACCAGGCGACATA CCAACGTAATAAAGGTGGACTCCT		TCTTCCACCCTCGATACCAC CCGGATACTACGGAGATGGA
Smad5	ACTATTGAAAACACTAGGCGGCATA CACCTCCCCACCAACGTAGTA	<u>Mutagenesis of Smad7 promoter</u>	
		Mut 166-167	GAATTAACACCCACAATTACCCGATGGGAACATAGCTCTG
		Mut 187-188	GGGAACATAGCTCTGCCCGTAGATGCACGTCCC
		Mut 212-213	GCACGTCCCATCCAGACCCGCTGTGACATCAGATTC