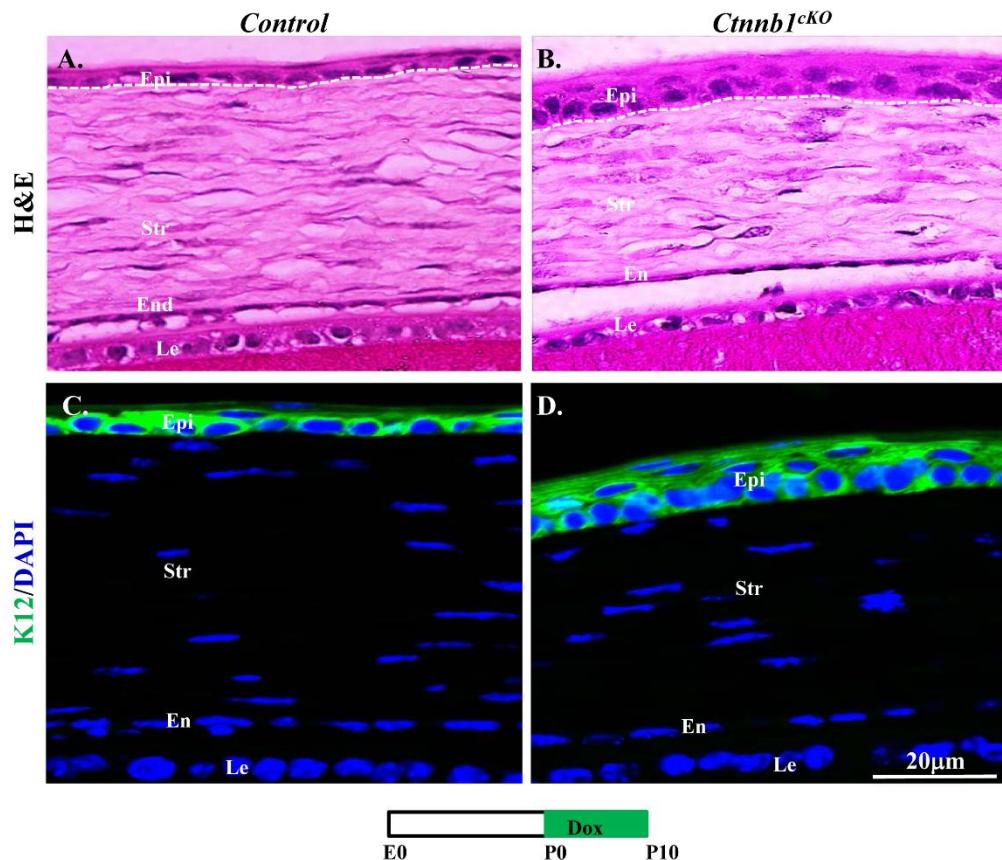
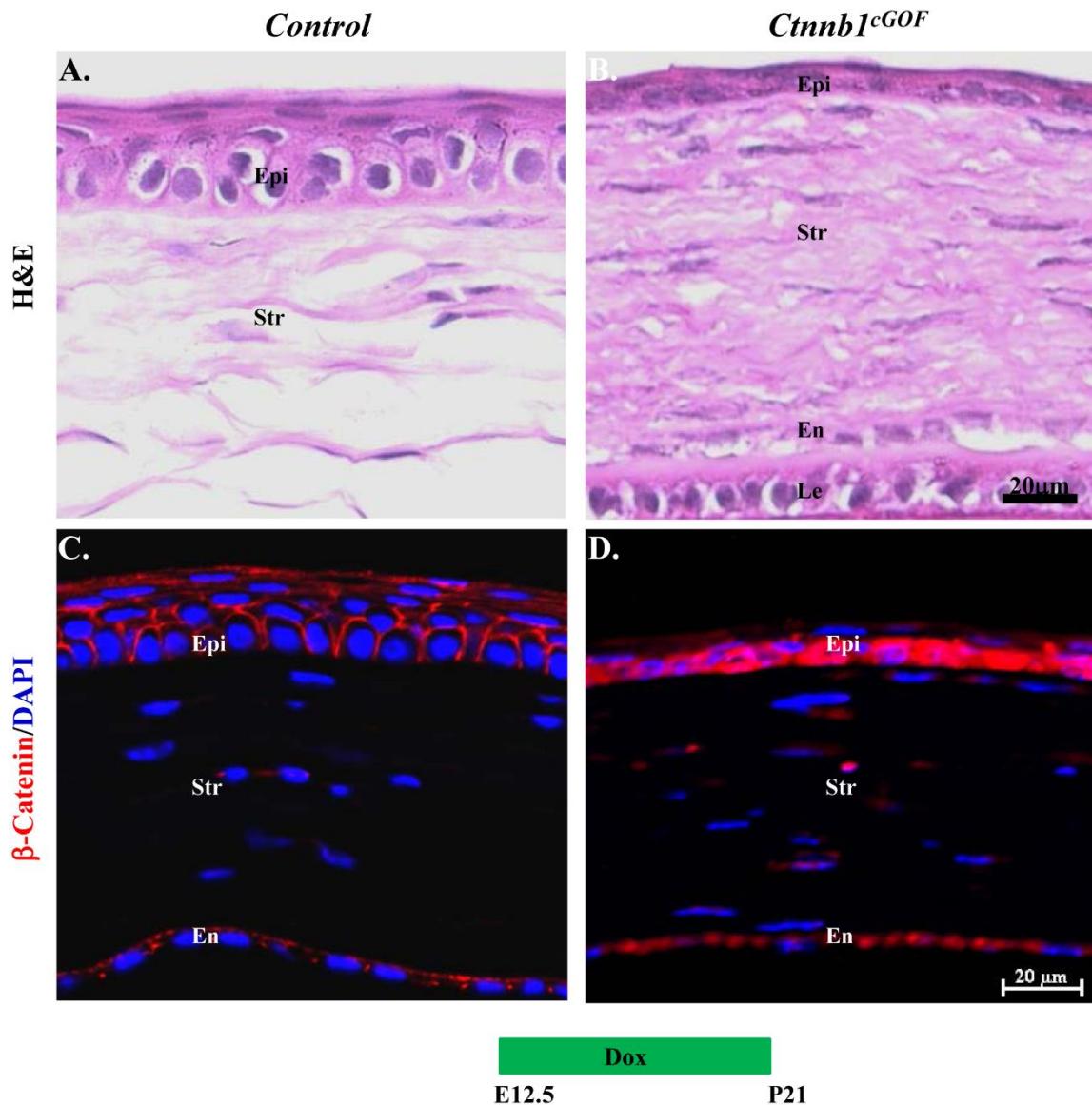


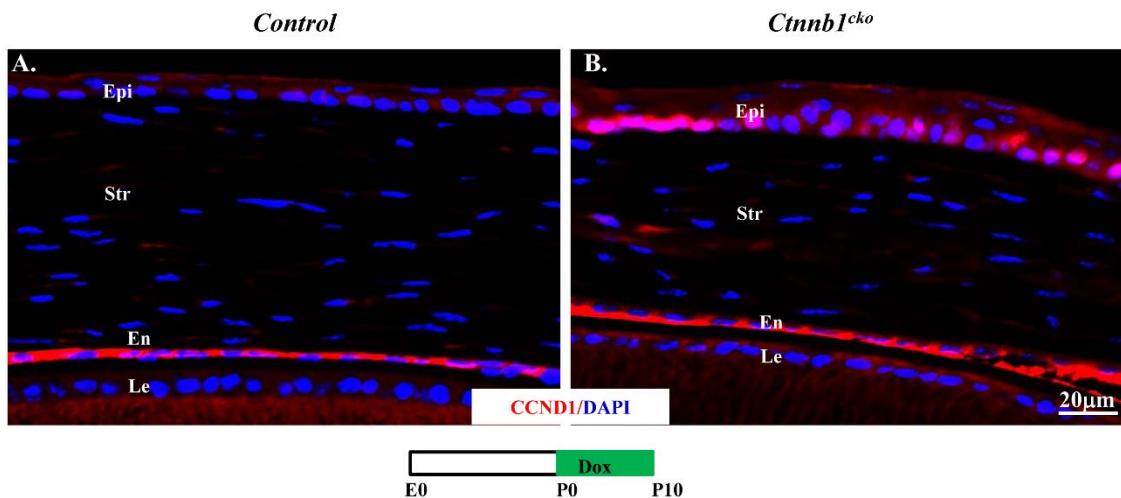
Supplementary Fig. 1. Wnt/β-catenin signaling activity is diminished during postnatal corneal morphogenesis in mice. (A-F) Whole mount X-gal staining (dark blue) of the *Axin2^{LacZ}* knock-in mouse corneas. Note strong activity displayed at P1 (A) and P2 (B) with a gradual reduction at P7 (C). At P15 (E) and P21 (F) no activity was detected. (G-I) 5 μ m paraffin sections of X-gal stained corneas. Strong X-gal staining (blue granules) was detected in the stroma at P1 then dramatically decreased at P10 and was absent at P21.



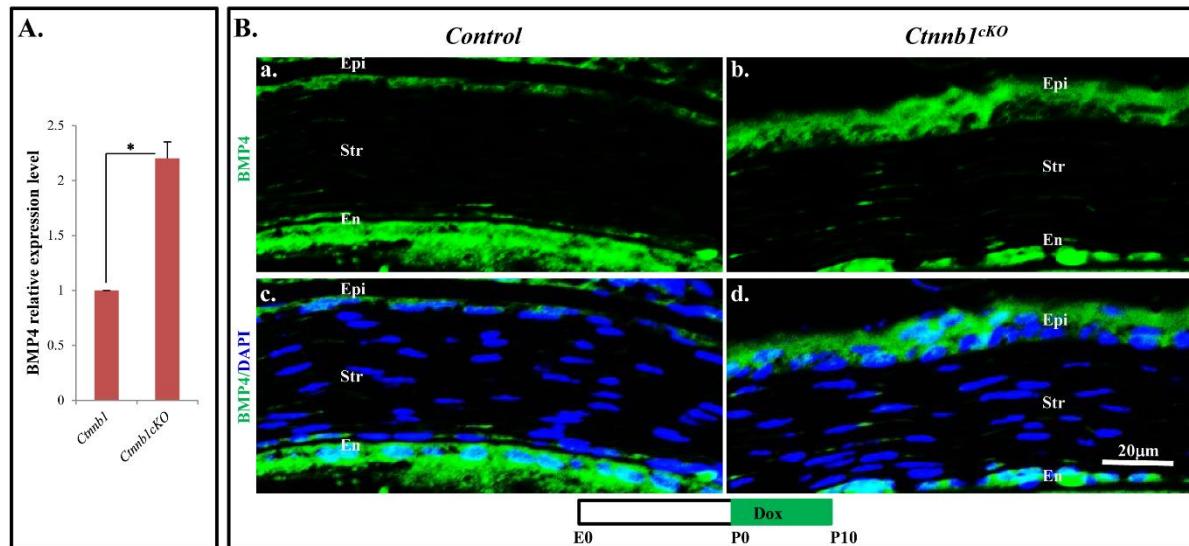
Supplementary Fig. 2. *Ctnnb1* is knocked out by Dox-induction from P0 to P10 and the cornea epithelium is keratin12 positive. H&E staining of the central cornea of the *Ctnnb1*^{cKO} mutant (B) and the littermate controls (A). Immunofluorescence staining showed that keratin 12 was expressed in control as well as *Ctnnb1*^{cKO} mutant. Abbreviations: epi: corneal epithelium; str, stroma; Le: Lens.



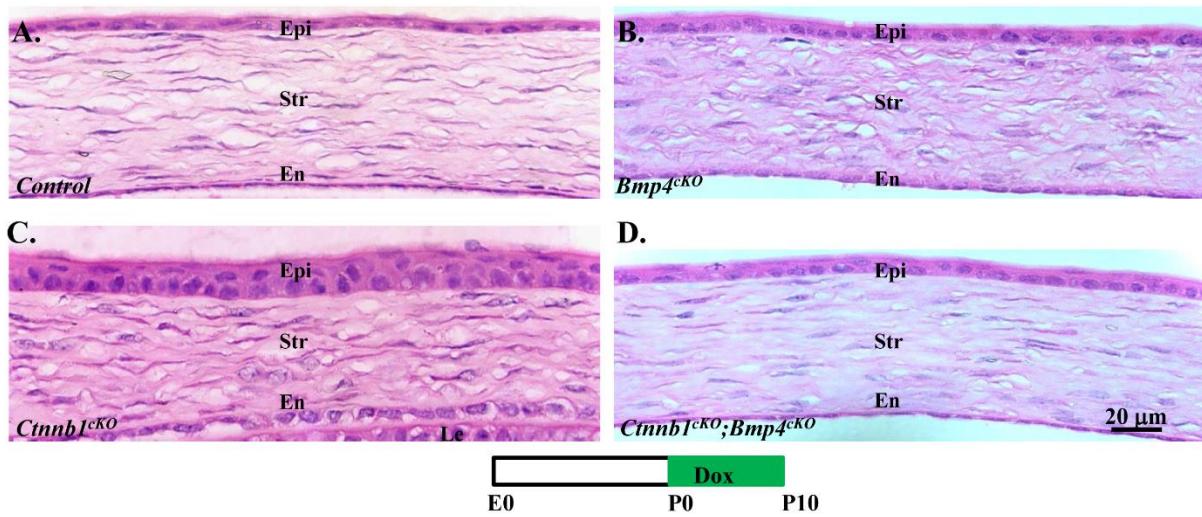
Supplementary Fig.3. Corneal epithelial stratification was retarded by expression of *Ctnnb1* gain of function mutant (*Ctnnb1^{cGOF}*) in keratocytes. (A, B) H&E staining results showed that corneal epithelium stratified to 5-6 cell layers in the control littermates (A), while only 1 or 2-cell layer epithelium was present in the *Ctnnb1^{cGOF}* mutant (B) at P21. (C, D) Immunofluorescence staining showed that β-catenin expression was increased in *Ctnnb1^{cGOF}* (D) mutant more than the control (C).



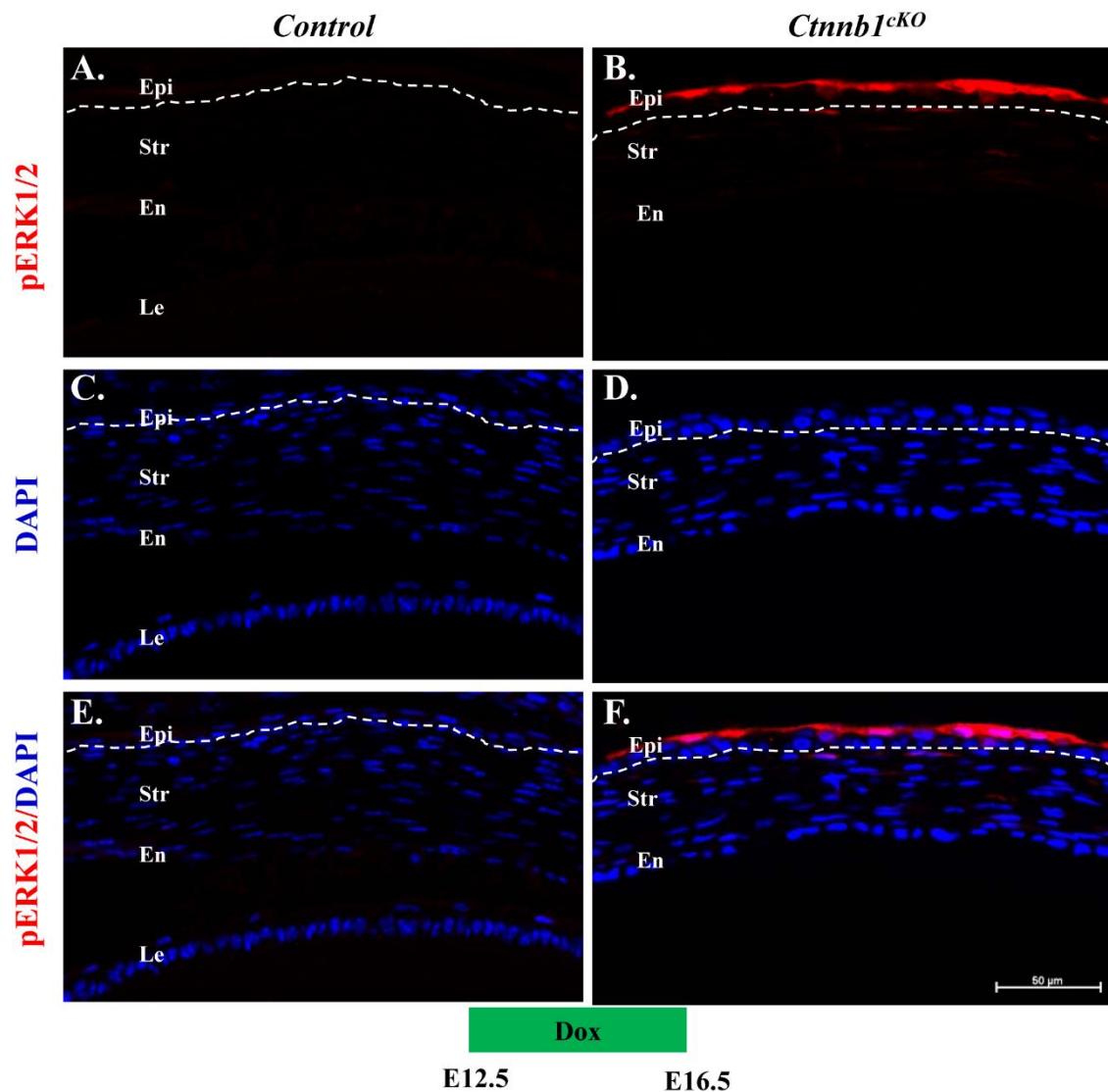
Supplementary Fig. 4. β -catenin ablation in the stroma up-regulated *CCND1* expression *in vivo*. Immunofluorescence staining showed that *CCND1* expression in *Ctnnb1^{cKO}* epithelium was increased. Abbreviations: epi: corneal epithelium; str, stroma; en, endothelium; le, lens.



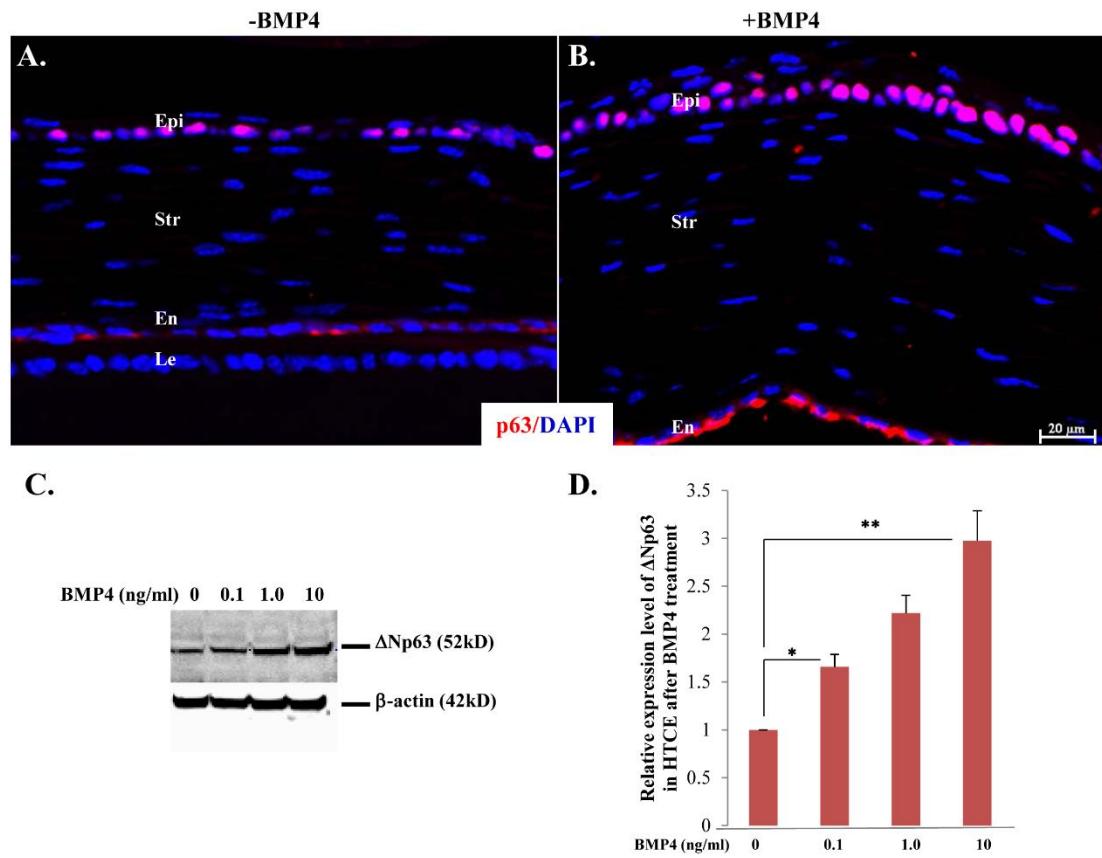
Supplementary Fig. 5. β -catenin ablation up-regulated BMP4 expression *in vivo* and *in vitro*. (A) qRT-PCR verified the increase of BMP4 after *Ctnnb1* ablation by infection of *Ad-Cre-GFP* in the primary cell cultures of stromal keratocytes isolated from *Ctnnb1^{ff}* mice at P0. (B) Immunofluorescence staining showed that BMP4 expression in both the stroma and the epithelium was increased in the absence of *Ctnnb1* (compare b,d to a,c). Abbreviations: epi: corneal epithelium; str, stroma; en, endothelium; le, lens. *P<0.05. Data are mean \pm s.e.m.



Supplementary Fig.6. *Bmp4* ablation in the stroma rescued the phenotype caused by knock-out of *Ctnnb1*. H&E staining revealed normal epithelial stratification in control (A) and *Bmp4*^{cKO} (B), consisting of 1 or 2-cell layers at P10. As expected, *Ctnnb1*^{KO} showed precocious epithelial stratification of 3 to 4-cell layers (C), which was counteracted by the simultaneous ablation of *Bmp4* in stromal keratocytes and resulted in normal epithelial cell stratification (D). Abbreviations: epi: corneal epithelium; str, stroma; en, endothelium; le, lens.



Supplementary Fig. 7. *Ctnnb1* ablation in keratocytes results in phosphoorylation of ERK1/2 (A-F) Immunofluorescence staining showed that at E16.5 ERK1/2 was phosphorylated in *Ctnnb1*^{cKO} mutant corneal epithelium compared with the littermate controls (compare A,C,E to B,D,F). Abbreviations: epi: corneal epithelium; str, stroma; en, endothelium; le, lens.



Supplementary Fig.8. Human recombinant BMP4 protein enhances p63 expression *in vivo* and in HTCE cells. (A, B) Immunofluorescence staining showed that p63 expression in corneal basal cells was increased by the subcutaneous injection of BMP4 protein (Compare B to A). (C) The expression of ΔNp63 in HTCE cells was raised after different dosages of BMP4 treatment. Lane 1: HTCE cells treated with PBS as control; lane 2: HTCE cells treated with 0.1 ng/ml BMP4 for 48 hrs; lane 3: HTCE cells treated with 1 ng/ml BMP4 for 48 hrs and lane 4: HTCE cells treated with 10 ng/ml BMP4 for 48 hrs. Abbreviations: epi: corneal epithelium; str, stroma; en, endothelium; le, lens. *P<0.05, **P<0.01. Data are mean ± s.e.m.

Supplementary Table S1. Primer Information for Transgenic Mouse Genotyping

Transgenic mouse line	PCR primer sequence	Note
KR	Forward: 5' TCAGCCATCGCTATGACTCAGTTC Reverse: 5' TTGTTCTCACGTGCCAGTACAGG	Mutant:408bp
TC	Forward: 5' GTCAGATGCCCTGGAGACGCC Reverse: 5' TCGCGAACATCTCAGGTTCTGC	Mutant: 320bp
Ctnnb1^{flx}	Forward: 5' AAGGTAGAGTGATGAAAGTTGTT Reverse : 5' CACCATGTCCTCTGTCTATTG	WT:233bp Mutant:300bp
Lrp5^{flx}	Common-S2: 5' CCACCAATCATCAGCCAAGGAAA Wt-AS2: 5' TCACCTGTCCTAGTGCAGAAGGAT Neo-AS2: 5' GCTTCCTCGTGCTTACGGTATC	WT: 309bp Mutant: 166bp
Lrp6^{flx}	Forward: 5' GGGTTCTACTTTGTGTGGTT Reverse: 5' CCTCCAAGCCTCCAACATACAATC	WT: 412bp Mutant: 468bp
BMP4^{flx}	Forward: 5' GAGCTAAGTTTGCTGGTTGC Reverse: 5' GCCCATGAGCTTTCTGAGA	WT: 200bp Mutant: 250bp
Axin2^{LacZ}	Forward: 5' AAGCTGCGTCGGATACTTGAGA Reverse: 5' AGTCCATCTTCATTCCGCCTAGC	WT: 400bp Mutant: 493bp
Ctnnb1^{flx}E3	pgkpr-spe(-):5' GACTAGTGAGACGTGCTACTT bCatE3(+): 5' CGCAAGAGCAAGTAGCTGGTAA bCatE4(-): 5' AGTTCCCGCGTCATCCTGATAGT	WT:368bp Mutant:707bp

Supplementary Table S2. List of antibodies used in this study

Primary antibody	Host	Source	Application
Anti-b-catenin (D13A1)	Rabbit	Cell signaling (#8814)	IHC (1:200)
Anti-b-catenin	Rabbit	BD Biosciences (#610153)	IHC (1ug/ml)
Anti-PCNA (PC10)	Mouse	Abcam, Inc (ab29)	IHC (1:200)
Anti-Bmp4	Rabbit	Abcam, Inc (ab39973)	IHC (1:200); WB(1:1000)
Anti-phopho-ERK1/2	Rabbit	Neuromics (RA15002)	IHC (0.1mg/ml);
Anti-ERK2 (E460)	Rabbit	Abcam (ab32081)	WB(0.1mg/ml)
Anti- Krt12	Rabbit	Custom made	WB(0.1ug/ml)
Anti-cyclin D1 (92G)	Rabbit	Cells Signaling (#2978)	IHC(1ug/ml)
Anti-b-actin	Gost	Santa Cruz biotec Inc (SC-1616)	IHC (1mg/ml)
Anti-TCF4(C48H11)	Rabbit	Cell signaling (#2569)	WB (1:200)
Anti-phopho-Smad1/5 (ser463/465) (41D10)	Rabbit	Cell Signaling (#9516)	ChIP (2ug/ml)
Anti-Smad1(D59D57)	Rabbit	Cell Signaling (#6944)	WB(1:500) WB(1:500)
Secondary antibody			
Anti-rabbit IgG Alexa488	Goat	Invitrogen, Inc.	IHC(1:500)
Anti-rabbit IgG Alexa555	Goat	Invitrogen, Inc.	IHC(1:500);WB(1:1000)
Anti-mouse IgG Alexa555	Rabbit	Invitrogen, Inc.	IHC(1:500)
Anti-goat IgG Alexa555	Donkey	Invitrogen, Inc.	IHC(1:500);WB(1:1000)

Supplementary Table S3. Primer information for the ChIP assay and RT-qPCR

Primer name	Sequence	Application	PCR product (bp)
mBMP4pr-F1 mBMP4pr-R1	5' GGCCAAAGGAAAGTCTTGTC 5' GGCTCCCAAGTTATCAGATG	LEF/TCF putative binding site 1 in mouse Bmp4 promoter region	102
mBMP4pr-F2 mBMP4pr-R2	5' GTTAGTCACGCAAACACAG 5' AAGCTTCTAAAGGAAGGGATTG	LEF/TCF putative binding site 2 in mouse Bmp4 promoter region	103
mBMP4pr-F3 mBMP4pr-R3	5' CGAGGTCCATCACTTATGAG 5' TCCAGCACCATGGCTAACTT	LEF/TCF putative binding site 3 in mouse Bmp4 promoter region	232
mBMP4pr-F4 mBMP4pr-R4	5' GTGCTCAGCCTCTAACACTC 5' GCCGCTCATCTGACCTTTGT	LEF/TCF putative binding site 4 in mouse Bmp4 promoter region	139
mBMP4pr-F5 mBMP4pr-R5	5' ATACTCTTAGCCTGGCTCAC 5' CTTCCAGGAAGGTAACACAG	LEF/TCF putative binding site 5 in mouse Bmp4 promoter region	288
mBMP4pr-F6 mBMP4pr-R6	5' TCCCGTGTATGATGAAGTC 5' GCATGCACCCAGGGTAAC	LEF/TCF putative binding site 6 in mouse Bmp4 promoter region	158
BMP4-F1 BMP4-R1	5' CGAGCCAACACTGTGAGGAG 5' CCGAGGAGATCACCTCATTC	RT-qPCR to detect mouse Bmp4 expression	130
GAPDH-F1 GAPDH-R1	5' GTGGTGAAGCAGGCATCTGAG 5' TTACTCCTGGAGGCCATGTAG	RT-qPCR to mouse GAPDH expression	228