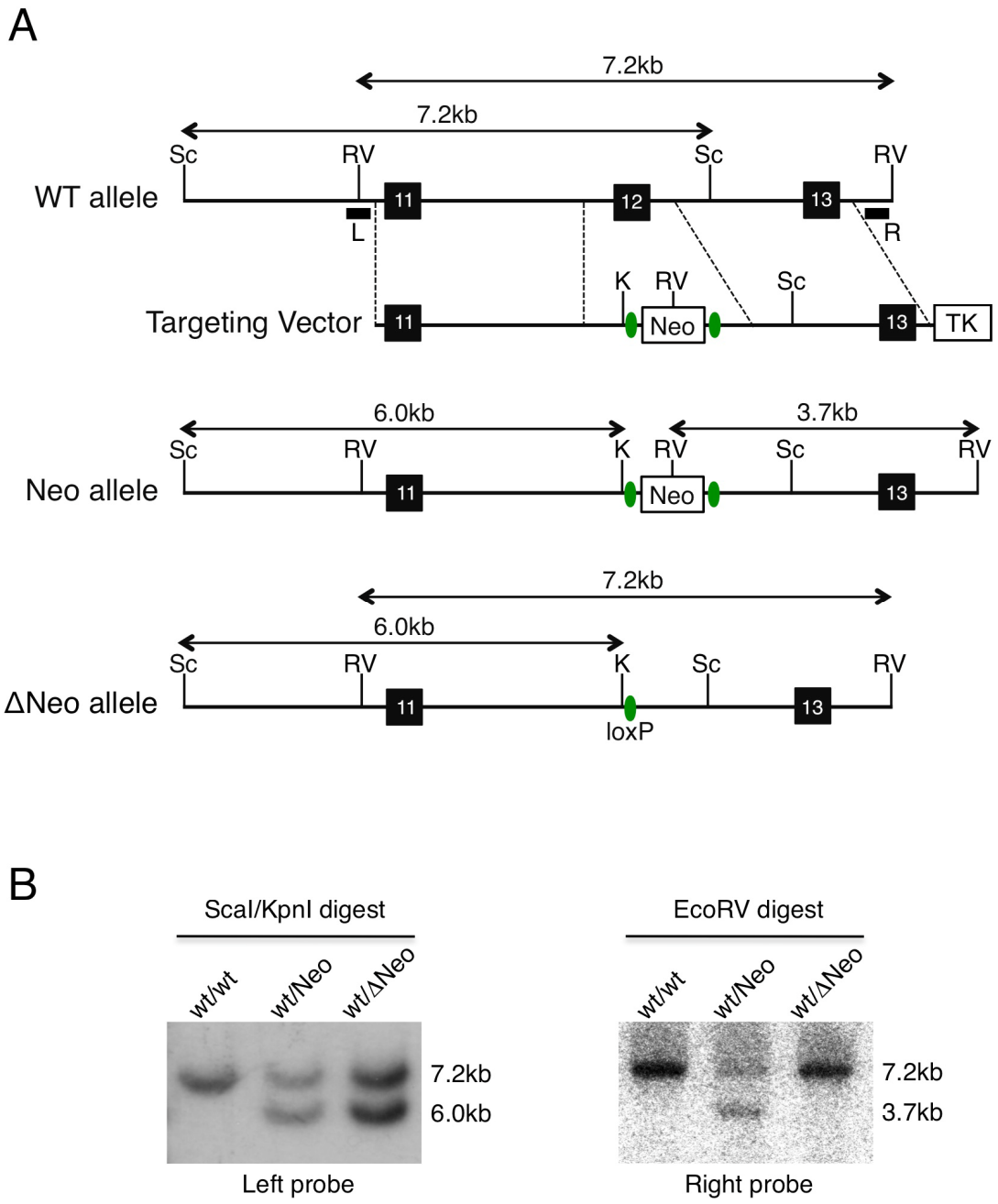

Fig. S1. Targeting of exon 12 in the *p63* gene by homologous recombination.

(**A**) Targeting strategy. Targeting vector consists of a 3.2 kb homologous left arm, a *loxP*-flanked neomycin resistant cassette (Neo), a 2.3 kb homologous right arm and a thymidine kinase cassette (TK). The neomycin cassette was removed from the Neo allele by crossing heterozygous mutant mice with Actβ-Cre transgenic mice, resulting in the generation of heterozygous p63C$^{+/-}$ mice harboring a ΔNeo allele. Solid lines

indicate the position of the left (L) and right (R) probes used for Southern blot analysis. Ovals in *green* dictate *loxP* sites. *Sc*, ScaI; *RV*, EcoRV; *K*, KpnI. (**B**) Southern blot analysis. Genomic DNA was digested with a combination of ScaI and KpnI (left panel) and EcoRV alone (right panel) and the membranes were hybridized with [^{32}P]dCTP-labeled probes.

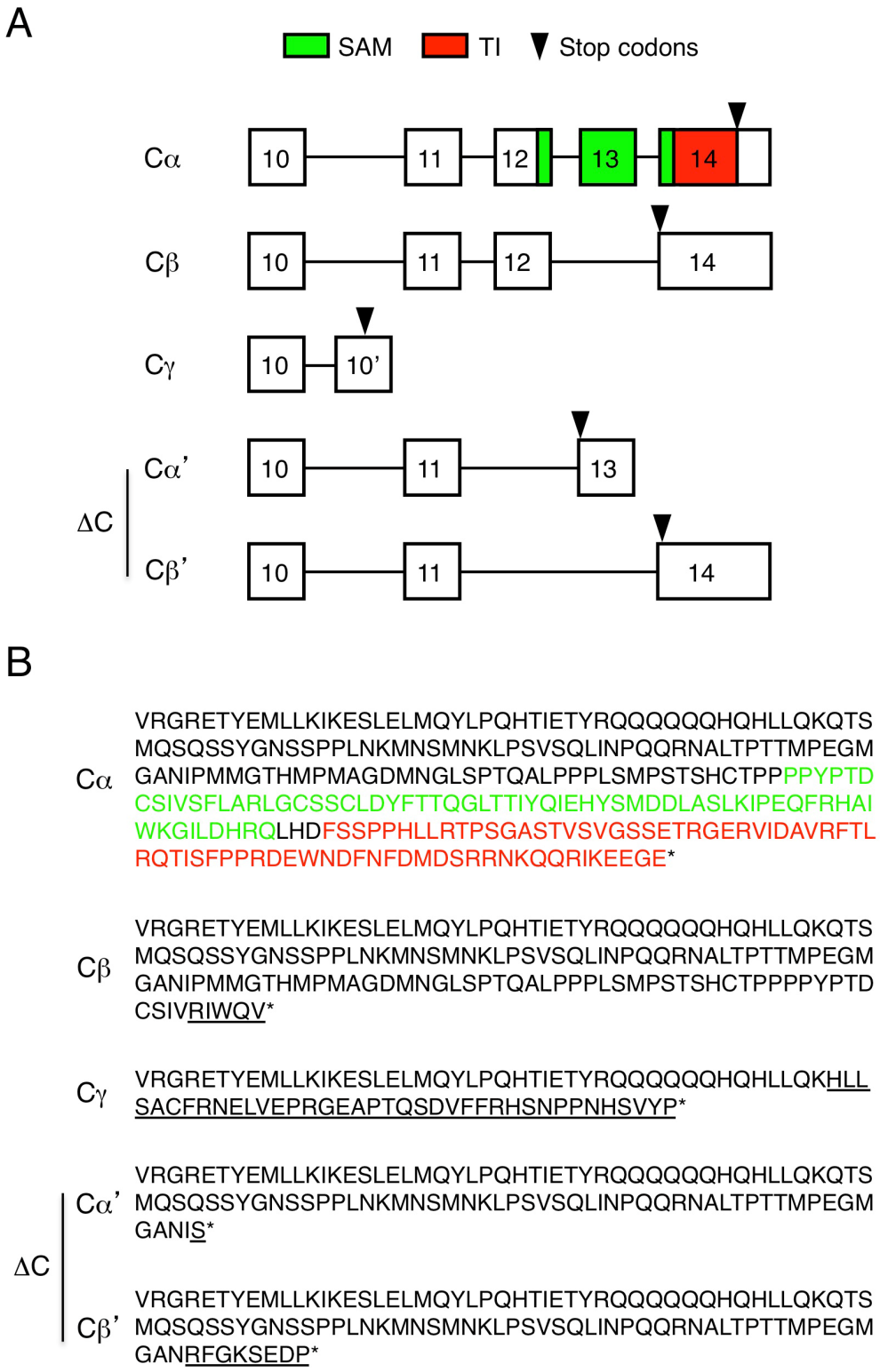

Fig. S2. Splicing and amino acid sequences of the C-terminus of p63 isoforms.

(**A**) Exon-intron structures at the C-terminus of p63 isoforms. WT mice express Cα, Cβ and Cγ isoforms while p63C$^{-/-}$ mice express Cα' and Cβ' isoforms as well as Cγ

isoform. (**B**) Amino acid sequences of the C-terminus of each p63 isoform. Sequences prior to exon 10 are not shown. *Green* and *red* sequences correspond to the $p63^{SAM}$ and $p63^{TI}$ domain, respectively. Asterisks indicate stop codons. Sequences unique to each isoform as compared to Cα are underlined. Note that Cα' and Cβ' isoforms differ by only 1 and 8 amino acids at its C-terminus and, therefore, they were not distinguished in this study and are collectively referred to as ΔC isoform.

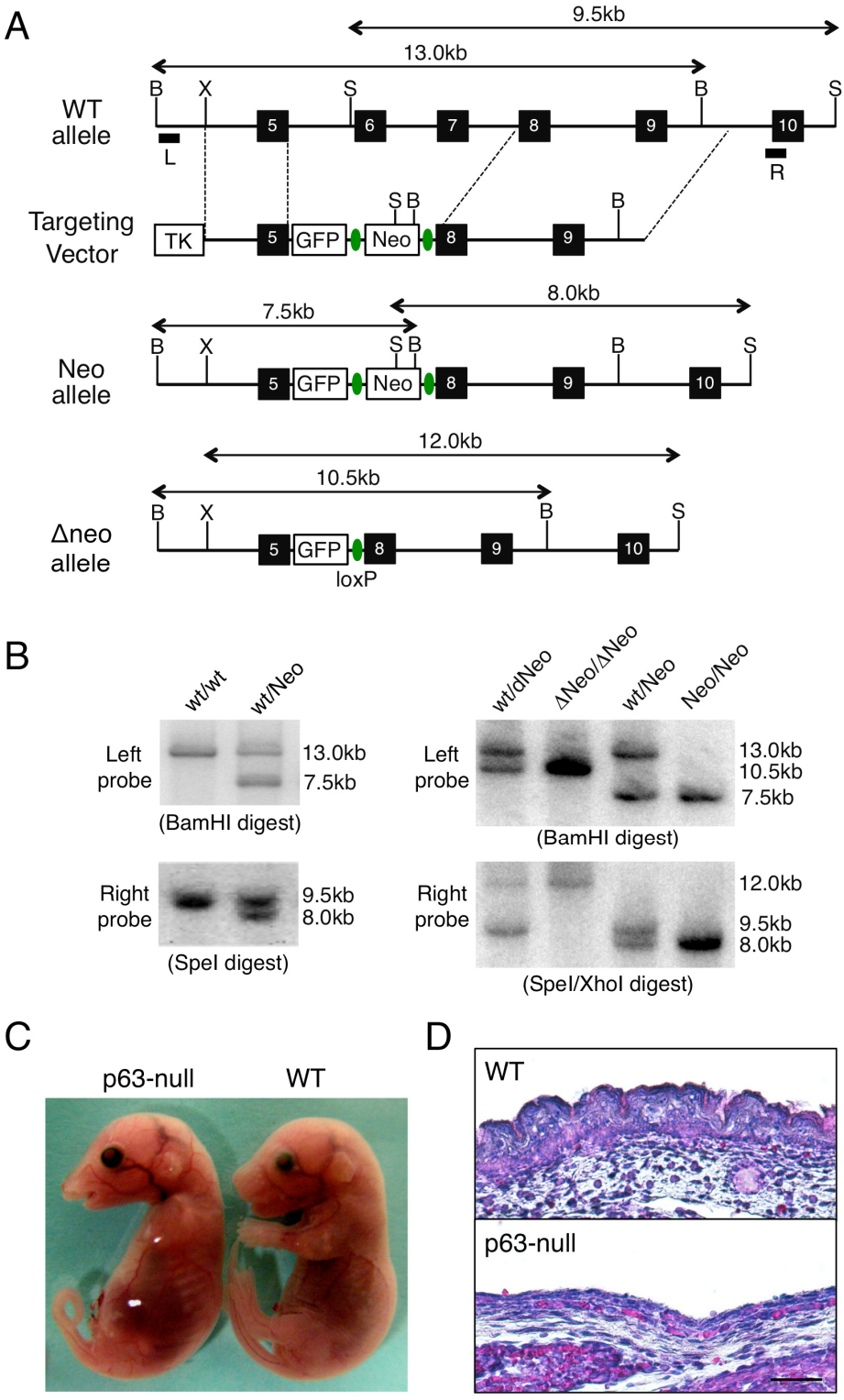

Fig. S3. Generation of p63-null mice by homologous recombination.

(**A**) Targeting strategy. Targeting vector consists of a thymidine kinase cassette (TK), a 5.0 kb homologous left arm, green fluorescent protein (GFP) coding sequences, a

loxP-flanked neomycin resistant cassette (Neo) and a 4.2 kb homologous right arm. The neomycin cassette was removed from the Neo allele by crossing heterozygous mutant mice with Actβ-Cre transgenic mice, resulting in the generation of heterozygous $p63^{+/-}$ mice harboring a ΔNeo allele. Solid lines indicate the left (L) and right (R) probes used for Southern blot analysis. Ovals in *green* indicate *loxP* sites. *B*, BamHI; *X*, XhoI; *S*, SpeI. (**B**) Southern blot analysis. Genomic DNA was digested with restriction enzymes and the membranes were hybridized with the left probe (top panels) and the right probe (bottom panels). (**C**) Gross appearance of p63-null and WT littermate mice at E17.5. Phenotypes of these p63-null mice are identical to those reported previously (Mills et al., 1999; Yang et al., 1999). (**D**) H&E staining of E17.5 WT and p63-null mouse epidermis. Note that p63-null mice are devoid of epidermis at this stage of development. Scale bar=50 μm.

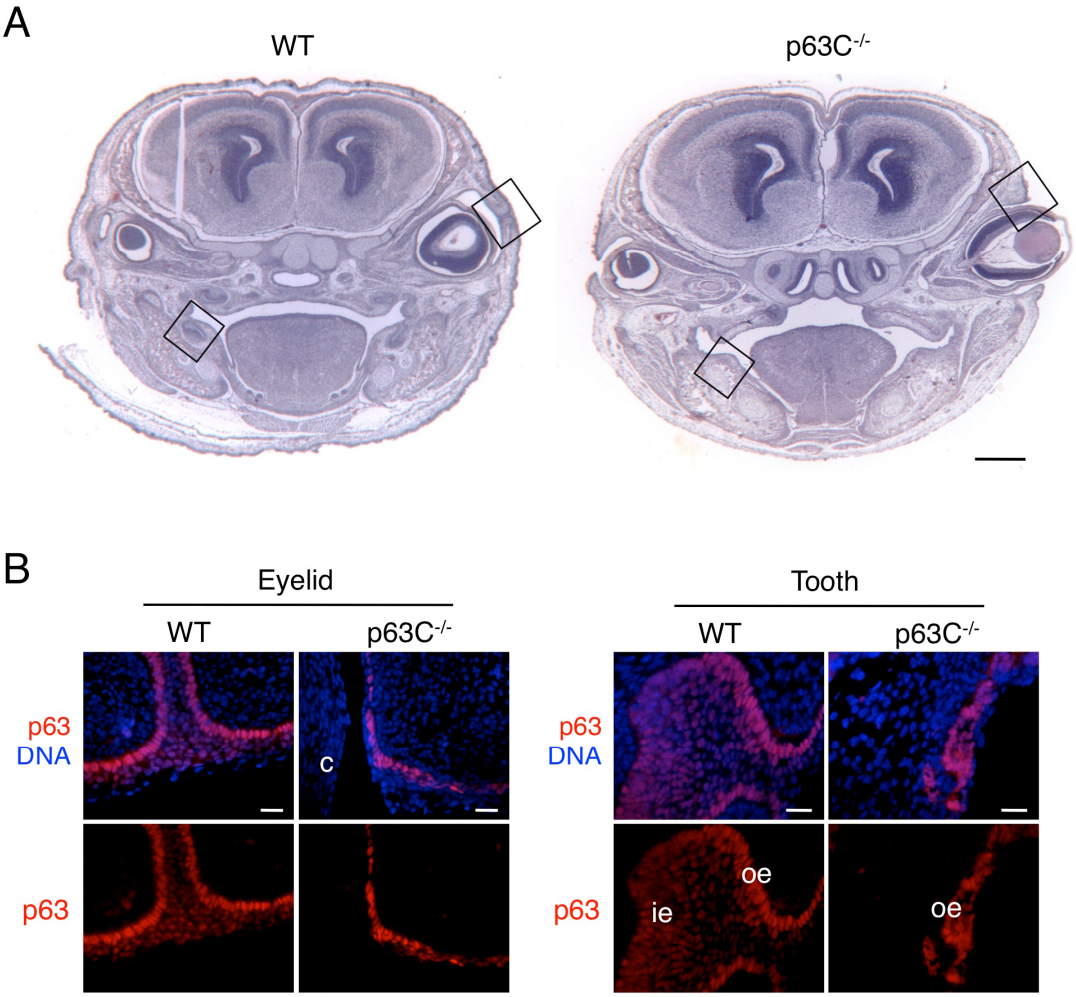

Fig. S4. Reduced expansion of epithelial progenitor cells in the eyelid and tooth of p63C$^{-/-}$ mice.

(**A**) H&E staining of coronal sections of the head of E17.5 WT and p63C$^{-/-}$ mice. Note that p63C$^{-/-}$ mice show poor development of molars and eyelids (boxed and highlighted in (**B**)) and palatal clefting (highlighted in Fig. 2B). Scale bar=500 µm.

(**B**) Immunofluorescence of E17.5 WT and p63C$^{-/-}$ mouse eyelid and molar tooth epithelia with anti-p63 antibody, counterstained with Hoechst 33342. Note that while WT mouse eyelids are fused at the midline supported by the expansion of p63-positive epidermal progenitor cells, eyelid epithelium of p63C$^{-/-}$ mice remains thin and does not cover the cornea. In WT molars, p63-positive cells have increased cellularity

and formed inner and outer enamel epithelia. In contrast, p63C$^{-/-}$ molars contain fewer p63-positive cells and remain as thickened dental epithelia. Scale bars=50 μm. *c*, cornea; *ie*, inner enamel epithelium; *oe*, outer enamel epithelium.

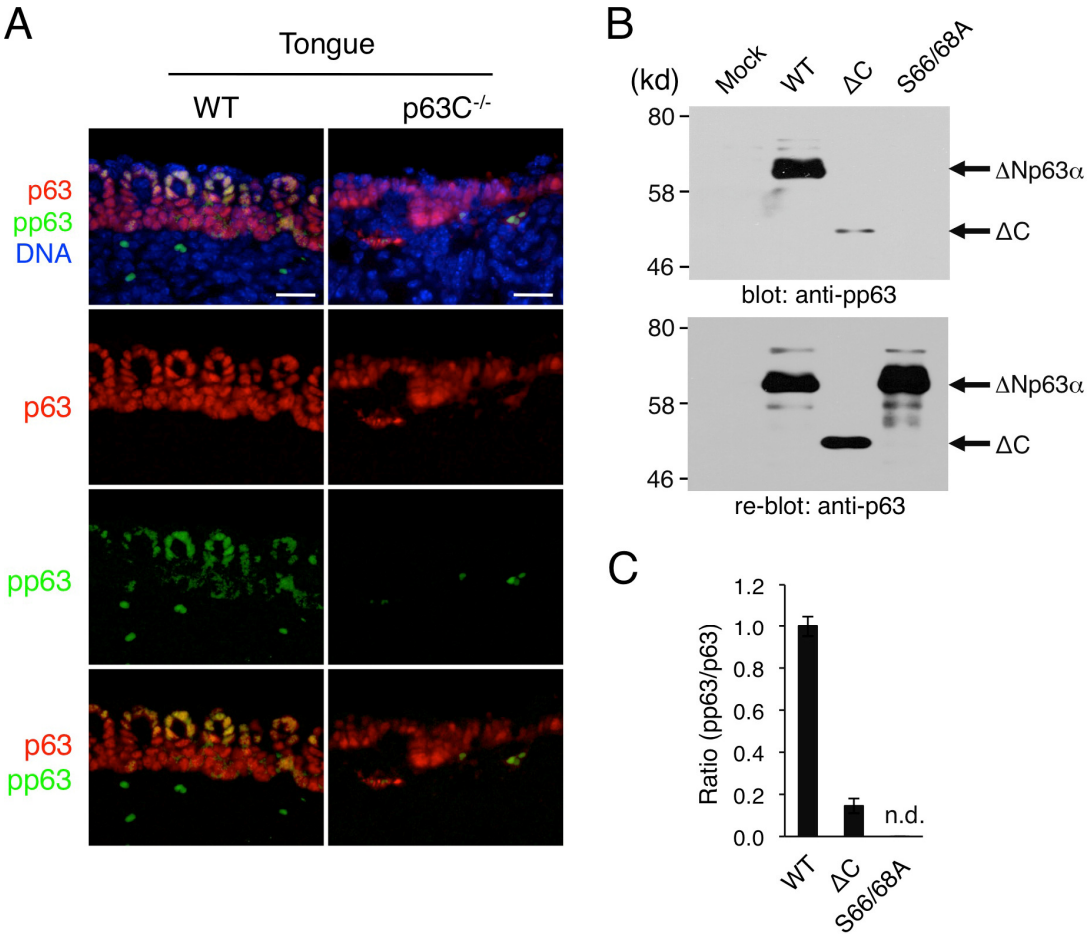

Fig. S5. Reduced p63 phosphorylation in the tongue epithelium of p63C$^{-/-}$ mice and validation of the specificity of anti-pp63 antibody.

(**A**) Immunofluorescence of E17.5 WT and p63C$^{-/-}$ mouse tongue epithelium with anti-p63 and anti-phosphorylated p63 (pp63) antibodies, counterstained with Hoechst 33342. Note that p63C$^{-/-}$ epithelium shows significantly reduced levels of p63 phosphorylation (*green*). Scale bars=50 μm. (**B**) Validation of the specificity of anti-pp63 antibody. Full-length ΔNp63α (WT), ΔNp63ΔC and unphosphorylatable S66/68A mutant of ΔNp63α (Suzuki and Senoo, 2012) were transfected into HEK293T cells and Western blot was performed using whole cell extracts. The membrane was first probed with anti-pp63 antibody (top), stripped and then re-probed with anti-pan-p63 antibody (bottom). *kd*, kilodalton. (**C**) Quantification of (**B**)

showing that S66/68 phosphorylation is undetectable in S66/68A mutant of ΔNp63α. In this condition, S66/68 phosphorylation of ΔNp63ΔC isoform is significantly reduced compared to that of WT ΔNp63α isoform. *n.d.*, not detected.

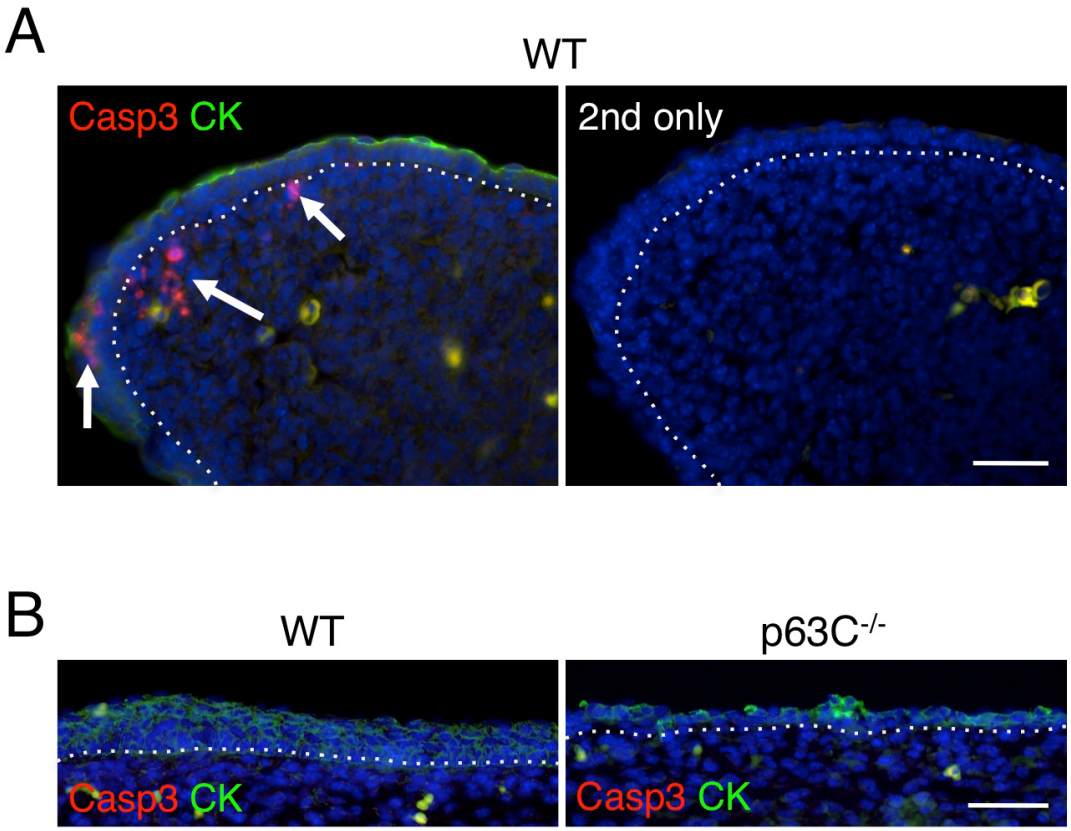

Fig. S6. No change in apoptosis of epidermal cells by loss of the p63 C-terminus. (**A**) Immunofluorescence of developing forelimbs of E13.5 WT mouse embryos with anti-cleaved caspase-3 (Casp3) and anti-pan-cytokeratin (CK) antibodies, counterstained with Hoechst 33342 (left panel). Some epidermal and mesenchymal cells in developing limbs at this stage undergo apoptosis as described previously (Ali-Khan and Hales, 2003), thus serving as a positive control for this marker (arrows). Right panel shows staining with secondary antibodies alone, serving as a negative control. Staining of these two slides was performed simultaneously with those shown in (**B**). Dotted lines indicate ectoderm-mesenchyme border. Scale bar=50 µm. (**B**) Immunofluorescence of E15.5 WT and p63C$^{-/-}$ mouse epidermis with anti-Casp3 and anti-CK antibodies, counterstained with Hoechst 33342. Note that Casp3-positive cells were undetectable in both WT and p63C$^{-/-}$ mice. Dotted lines indicate epidermal-dermal border. Scale bar=50 µm.

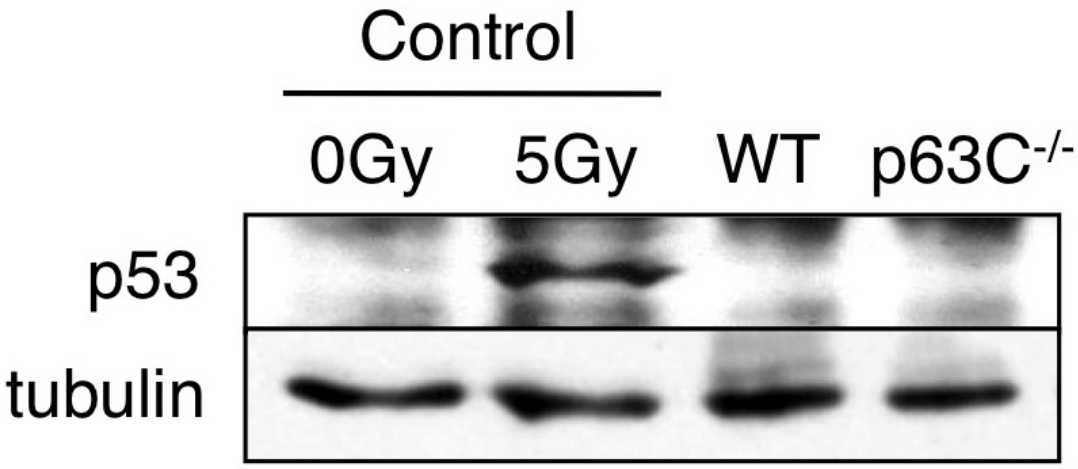

Fig. S7. No change in p53 expression in epidermal cells by loss of the p63 C-terminus.

Western blot of whole epidermal cell extracts from E15.5 WT and p63C$^{-/-}$ mouse embryos with anti-p53 antibody. Freshly isolated thymocytes untreated (-) or treated (+) with 5Gy γ-irradiation were used as a negative and a positive control, respectively. Anti-tubulin α antibody was used as a loading control.

Table S1. Primers used in this study

Primer	Sequence (5′-3′)	Product
Genotyping p63C$^{-/-}$ mice		
Neo allele	GGGTGGGGTGGGATTAGATAAATG and GAATGCTGTCTTGTGGTTGA	260 bp
Δ*Neo* allele*	AGGTATAGAATGCATGAAGG and GAATGCTGTCTTGTGGTTGA	350 bp
Cre transgene	TCCAATTTACTGACCGTACACCAA and CCTGATCCTGGCAATTTCGGCTA	540 bp
Genotyping *p63*-null mice		
Neo allele	GAGCTGAGCCGTGAGTTCAATGAGG and GGTGGATGTGGAATGTGTGCGAGGC	1.3 kb
Δ*Neo* allele	GAGCTGAGCCGTGAGTTCAATGAGG and GCAGATGAACTTCAGGGTCAG	174 bp
WT *p63* allele	AAGCATTGTTTCCTGTTTATTTCTGG and GACAAAGGCTATGACAGACAT	778 bp
qPCR		
TAp63-forward	TACTGCCCCGACCCTTACAT	
TAp63-reverse	GCTGAGGAACTCGCTTGTCTG	
ΔNp63-forward	TGCCCAGACTCAATTTAGTG	
ΔNp63-reverse	TGGAGCTGGGCTGTGCATAG	
Rps18-forward	CATGCAGAACCCACGACAGTA	
Rps18-reverse	CCTCACGCAGCTTGTTGTCTA	
p21$^{WAF1/CIP1}$-forward	CGATGGAACTTCGACTTTGTCA	
p21$^{WAF1/CIP1}$-reverse	GCACAAGGGTACAAGACAGTG	
GAPDH-forward	AGCCACATCGCTCAGACAC	
GAPDH-reverse	GCCCAATACGACCAAATCC	
p63 C-terminus splicing		
Exon 11-forward	GAACAGCATGAACAAGCTGCC	
Exon 14-reverse	GCTCATTCTCCTTCCTCTTTG	
Exon 6-forward	GCCCAGTATGTAGAAGATCC	
Exon 12/14-reverse	TCAGACTTGCCAAATCCTGAC	
Exon 10'-reverse	CTATGGGTACACGGAGTGGT	

*This primer pair also amplifies the WT *p63* allele (461 bp).