Supplemental data

p63 control of desmosome gene expression and adhesion is compromised in AEC syndrome.

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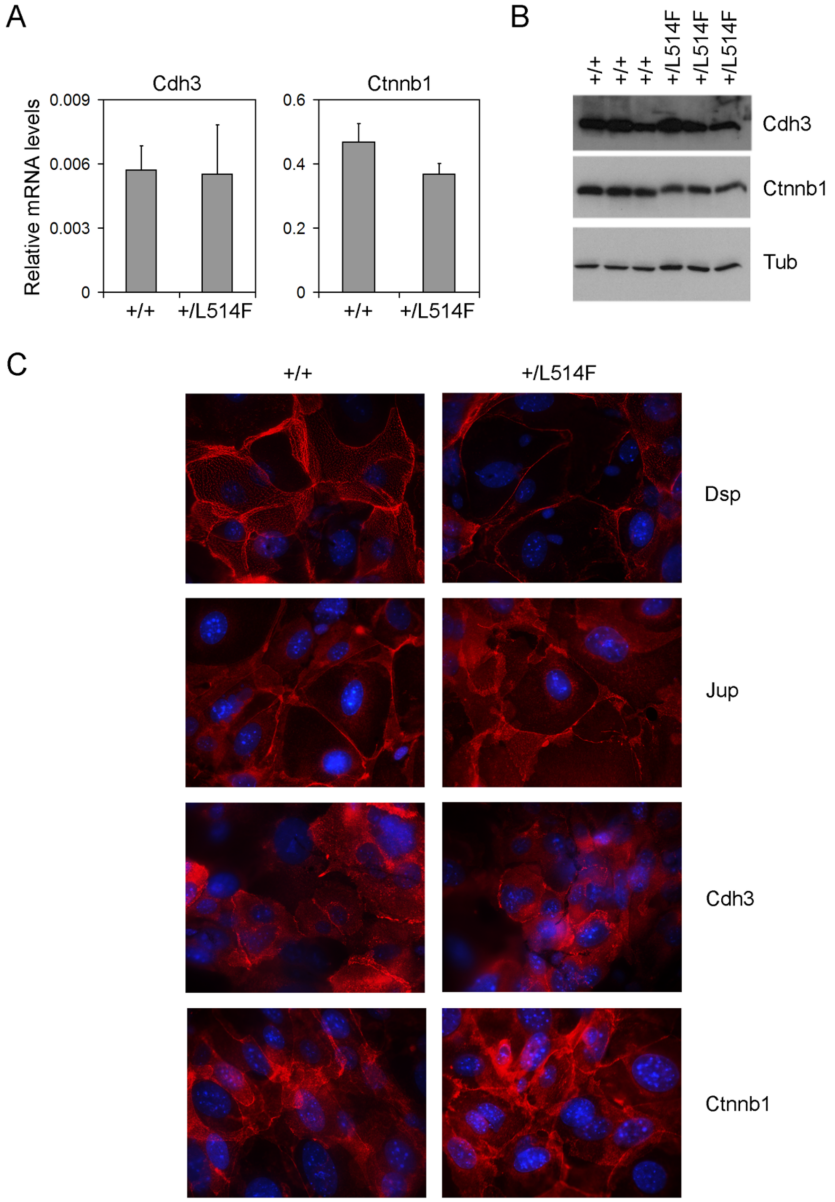
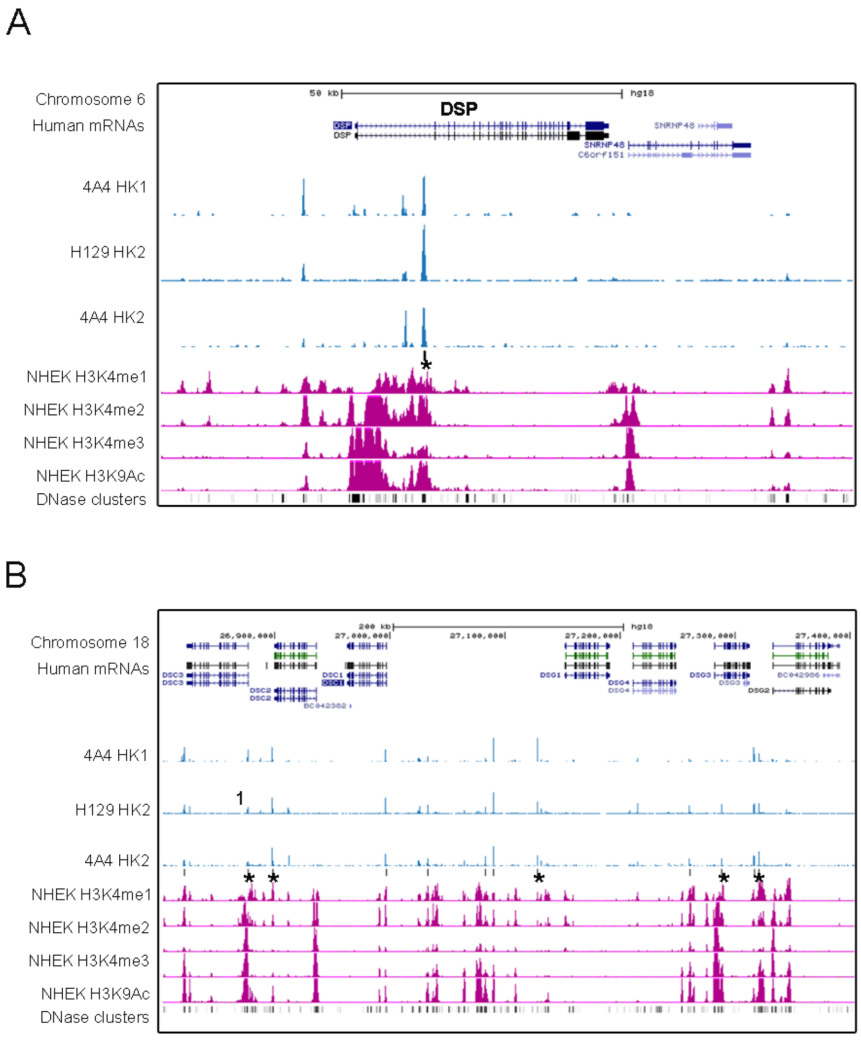
Supplemental Figures

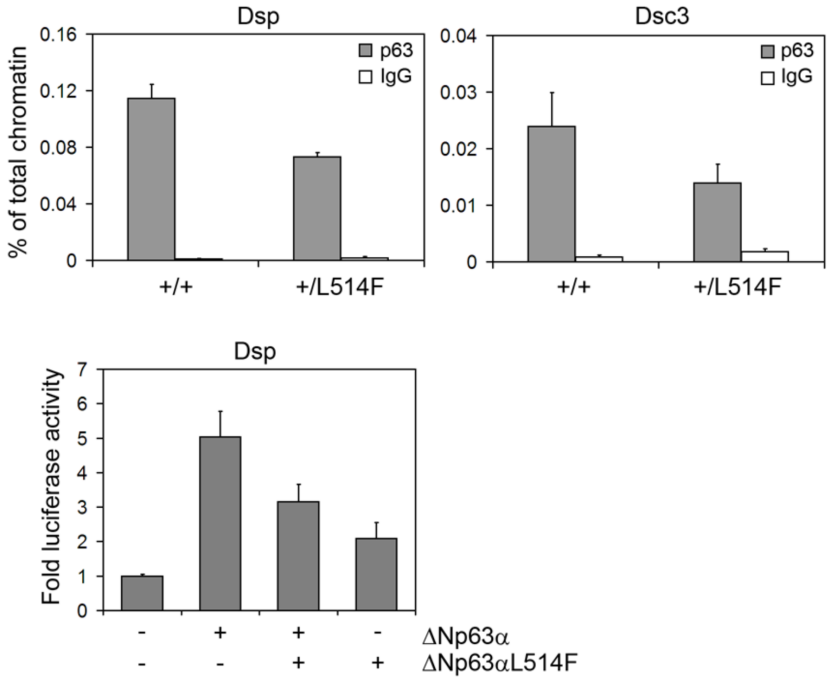


Supplemental Figure 1. Cell adhesion components are not affected in AEC mutant keratinocytes and epidermis. (A) Real time RT-PCR on RNA isolated from embryonic skin at E.14.5 from mutant (white bars) and control embryos (grey bars). Data are normalized for ß-actin mRNA levels, and are represented as mean of three independent biological samples +/- SD. (B) Immunoblotting of the indicated adherens junction proteins in wild-type (+/+) and AEC mutant (+/L514F) newborn epidermis. Tubulin antibodies (Sigma) were used as normalization control. (C) Immunofluorescence for the indicated adherens junction and desmosomal proteins

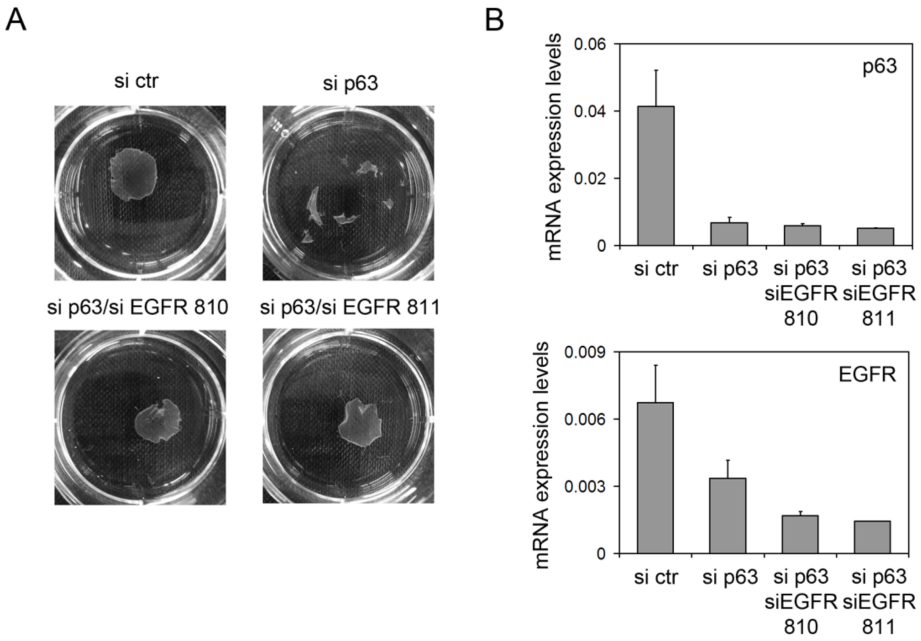
was performed on methanol fixed differentiated primary keratinocytes derived from wt and +/L514F mice.



Supplemental Figure 2. The position of the p63 binding regions in the *Dsp* and in the desmosomal cadherin genomic loci was obtained in human primary keratinocytes by ChIP-seq using 4A4 and H129 antibodies (56). Genomic regions enriched for H3K4me and DNAse clusters are ENCODE consortium data (57, 58). p63-binding regions corresponding to the mouse genomic regions tested in this study are indicated with *.



Supplemental Figure 3. AEC mutations affect both DNA binding and transactivation activities. (A) ChIP assay from wild-type and p63+/L514F embryonic skin (E14.5) was performed using p63 polyclonal antibodies (H-129) (grey bars) and rabbit IgG (white bars) as negative control. A reduction in p63 binding was observed in mutant skin compared the wild-type counterpart. Error bars denote SEM. These data are representative of two independent experiments (B) Luciferase assay in H1299 cells transfected with ΔNp63alpha or ΔNp63alphaL514F expressing vectors and Dsp luciferase reporter. Error bars denote SEM.



Supplemental Figure 4. EGFR knockdown restores mechanical resistance to p63 depleted keratinocytes. (A) Wild-type mouse keratinocytes were transfected with siRNA for p63 (sip63) and EGFR (EgfrMSS203810, EgfrMSS203811, Invitrogen) or control (siCTR) as indicated. Twenty-four hours after transfection, cells were treated with 0.6mM calcium for additional 24 hrs. Cells were detached as a monolayer with dispase. Monolayers were subjected to mechanical stress, and resulting fragments of the cell sheet were imaged. (B) p63 (upper panel) and EGFR (lower panel) expression were measured by real time RT-PCR on RNA isolated from samples in (A). Data are normalized for actin mRNA levels and are represented as mean +/- SD normalized mRNA levels.

Supplemental Methods

Immunofluorescence analysis on cells.

Mouse primary keratinocytes were seeded onto acid-washed collagen coating glass coverslips, grown for seven days and then treated with 0.6mM Ca2+ for 24h. Coverslips were rinsed in PBS and fixed in methanol. Indirect immunofluorescence was performed using anti Dsp (1:100), Jup (1:100), mouse anti ß-catenin (BD Transduction Laboratories; 1:100), and rat anti P-cadherin (Invitrogen; 1:50) antibodies. Secondary antibodies used for immunofluorescence staining were Alexa Fluor 488 goat anti-mouse, rabbit or chicken (Invitrogen). Images were captured using Zeiss Axioskop2 plus image microscope.

Supplemental tables

Table 1. p63 binding regions tested by ChIP.

Gene	Chrom.	Position of the p63-binding region	NCBI release
hDSP	6	7,498,979-7,499,303	NCBI36/hg18
mDsp	13	38,254,755-38,254,974	NCBI37/mm9
hDSC3	18	26,898,025-26,898,495	NCBI36/hg18
mDsc3	18	20,185,228-20,185,440	NCBI37/mm9
hDSC3(2)	18	26,876,703-26,877,063	NCBI36/hg18
mDsc3(2)	18	20,160,526-20,160,774	NCBI37/mm9
hDSG1	18	27,128,313-27,128,654	NCBI36/hg18
mDsg1	18	27,128,313-27,128,654	NCBI37/mm9
hDSG2	18	27315895-27316499	NCBI36/hg18
mDsg2	18	20,708,117-20,708,439	NCBI37/mm9
hDSG3	18	27287949-27288457	NCBI36/hg18
mDsg3	18	20,675,608-20,675,938	NCBI37/mm9

Table 2. Oligonucleotide primers for genotyping

Kb from TSS	Forward primer (5'-3')	Reverse primer (5'-3')
p63wt	GTCTGACCTCCCGACCCACCTCCT	GCATGATGAGCAGCCCAACCTTGCT
p63 mut	GTCTGACCTCCCGACCCACCTCCT	GCATGATGAGCAGCCCAACCTTGCA

Table 3. Oligonucleotide primers for Real Time RT-PCR on mouse samples.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
ß-actin	CTAAGGCCAACCGTGAAAAGAT	GCCTGGATGGCTACGTACATG
Dsc1	CTTTTCTTTCTCCTGGTTCTGGTATT	GAGGGAACGTGAAGGGAAACT
Dsc3	CCACCGTCTCTCACTACATGGA	TGTCCTGAACTTTCATTATCAGTTTGT
Dsg1	TCACCCCCTTTTTCATTATCTACTG	GTGGATTCTCCAAGTCTTGACCTT
Dsg3	TTTGCAAAACCCTGCAGAGA	CTGAAGTGATCTTGGCAATTGG
Dsp	CACCGTCAACGACCAGAACTC	GATGGTGTTCTGATTCTGATGTCTAGA
Pkg	TTCCGGCTCAACACCATTC	CGCTGGATGTTCTCCACAGA
Pkp3	CAAAAATGTTACAGGGATCCTATGG	GGCGAGGCGGTCCTTTAG
P63wt	GTCTGACCTCCCGACCCACCTCCT	GCATGATGAGCAGCCCAACCTTGCT

P63mut	GTCTGACCTCCCGACCCACCTCCT	GCATGATGAGCAGCCCAACCTTGCA
EGFR	CCACACTGCTGGTGTTGCTG	TTGGCAGACTTTCTTTTCCTCC
CTNNB1	TTAAACTCCTGCACCCACCAT	AGGGCAAGGTTTCGAATCAA
CDH3	CCATCACCACTCACCCAGAGA	CCTCAAAATCCAAACCCTTCTTG

Table 3. **Oligonucleotide primers for Real Time RT-PCR on human samples**

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
RPLP0	GACGGATTACACCTTCCCACTT	GGCAGATGGATCAGCCAAGA
Dsc1	GAGCATTGACCGTGAGAAATATGA	GCCATCTGCAGTTGTTGCAT
Dsc3	TGAAAATGACAACCACCCTGTTT	CCCCCACTGTAGTACCAGGTCTA
Dsg1	CCACCTGATAACGCAAATATAATTGA	TTGCATTTCTCTGCCACCATAC
Dsg3	CCTGCCGTATGGAGTATCACAA	GGTATCTGTTCCTGGGCTCTGA
Dsp	TGCGAGCAGAGCTCATCGT	CGAGTCAGTTGTATTCCATCTCCAT
Pkg	AGGAGAGCAAGCTGATCATCCT	CCAGAGCAGCTTTTCATAACTGTAGT

Table 4. Oligonucleotide Primers for ChIP analysis on mouse genomic DNA

SET	Kb from TSS	Forward primer (5'-3')	Reverse primer (5'-3')
Dsp	Intr.1	GCAGGGAGGAACCCCAAATA	CACACCACAGGAAAGCGTTTT
Dsc3	24 up	TCCAGGCGTCAAATGCATT	AGGACATGCAGGGTGTGGAT
Dsc3 (1)	5'UTR	GCCCAGGAGCGGTGTTC	CAGCGTCTCCTTGGTCCAA
Dsg3	Intr.1	CCTGCCTGGGAAGGTAGGA	TACAAGCTCTGAGGAGAATGAGTCTT
Dsg1c	18 up	TTTGCAAGGCCCAAACATG	CGGGACTTACTGCGGGTTAG
Dsg2	8 up	ATGCTGGTCTTTGAGCAGTTTCT	TGCCCTTACCTTGGCAACAT

Table 5. Oligonucleotide primers for pGL3-Dsc3-Luc and pGL3-Dsp-Luc cloning

SET	Forward primer (5'-3')	Reverse primer (5'-3')
Dsp-p63 BS Kpn F Dsp p63 BS SacI R	AGCTTGGTACCCCTGTACGTGT TAGGACAACAGAT	AGCTTGAGCTCGTACCCTGGGTT ACAAGCAAAAC
Dsc3-p63BS Kpn F Dsc3-p63BS SacI R	AGCTTGGTACCACATTCAGACA GACACACACACAC	AGCTTGAGCTCCCTTCCAGTTAC TTAGGCTTGAAA

Table 6. Oligonucleotide primers for mutagenesis of the p63-binding site in *Dsc3 and Dsp* regulatory regions

Kb from TSS	Forward primer (5'-3')	Reverse primer (5'-3')
24 up of Dsc3	CCTCACCCACACTCAG**GG**AGTG CTGGCTTGTTGG*	CCAACAAGCCAGCACTCCCTGAGT GTGGGTGAGG
11 down of Dsc3	CGTTATCCACACCCTG**GG**TGTCC TGACCTGCAGG*	CCTGCAGGTCAGGACACCCAGGGT GTGGATAACG

* underlined in bold is the 2bp mutation. The p63-binding core CTAG (Dsc3) and CATG (Dsp) was mutated in GGAG.