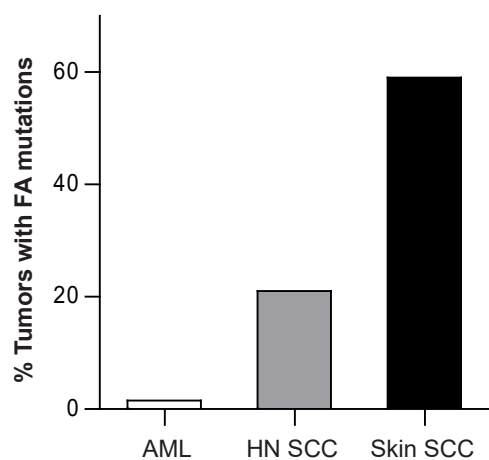


**Figure S1 Related to Table 1**

**A**



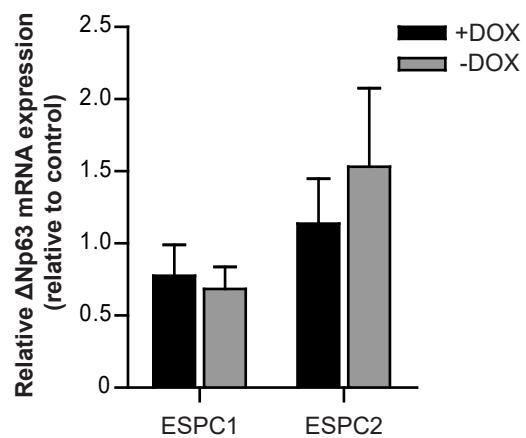
**B**

Gene	Mutations		
	AML	HN SCC	Skin SCC
FANCA	0%	1.1%	24%
FANCB	0%	1.1%	3%
FANCC	0.5%	0.7%	3%
FANCD1/BRCA2	0%	4%	34%
FANCD2	0%	1.1%	10%
FANCE	0%	0%	3%
FANCF	0%	0%	3%
FANCG	0%	0.7%	3%
FANCI	0.5%	1.1%	7%
FANCI/BRIP1	0%	1.8%	3%
FANCL	0%	0.4%	3%
FANCM	0.5%	1.1%	3%
FANCN/PALB2	0%	1.4%	7%
FANCO/RAD51C	0%	0.7%	0%
FANCP/SLX4	0%	1.8%	0%
FANCQ/ERCC4	0%	1.4%	7%
FANCR/RAD51	0%	0.4%	0%
FANCS/BRCA1	0%	2.9%	10%
FANCT/UBE2T	0%	0%	0%
FANCU/XRCC2	0%	0%	0%
FANCV/MAD2L2	0%	0.7%	0%
FANCW/RFWD3	0%	1.1%	0%

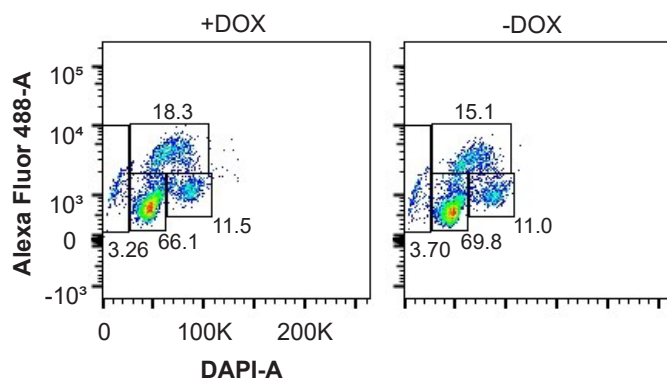


Figure S2 Related to Figure 1

**A**



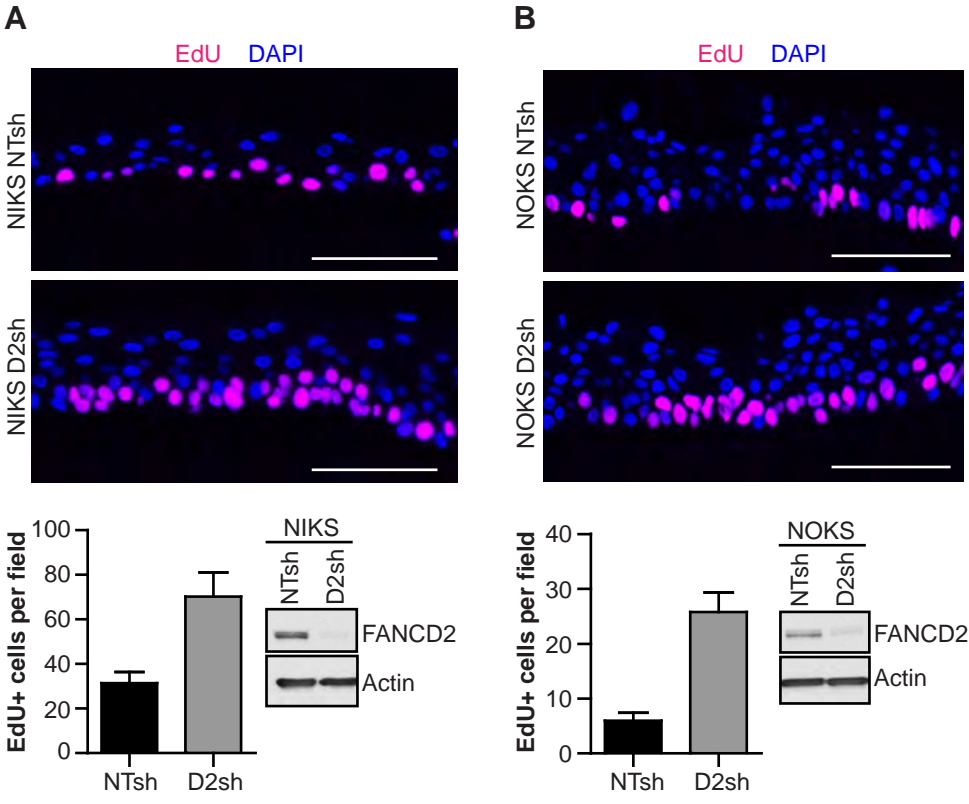
**B**



**Figure S2 Related to Figure 1: PSC-derived ESPCs exhibit basal cell characteristics. (A)**

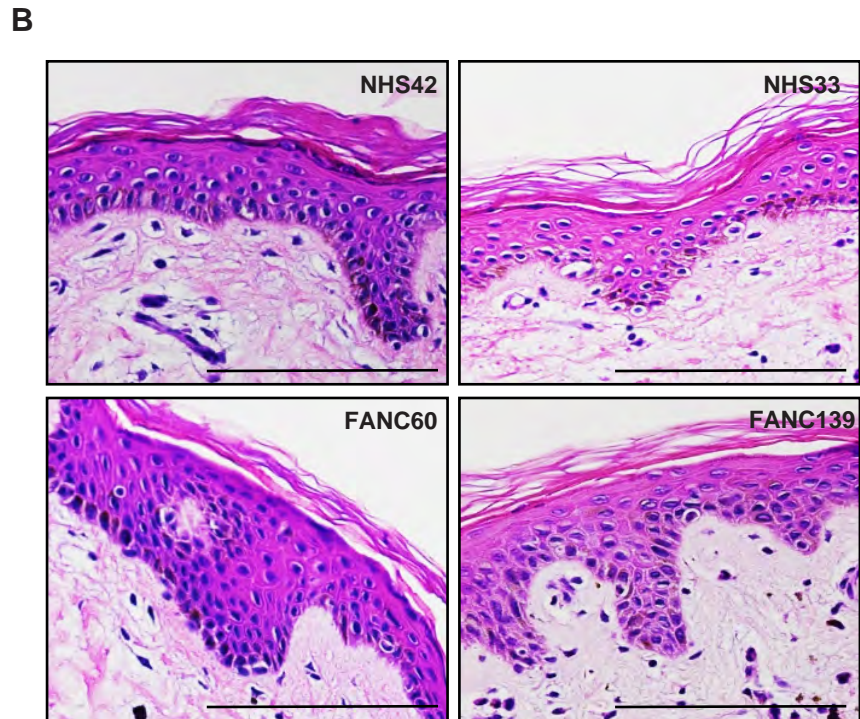
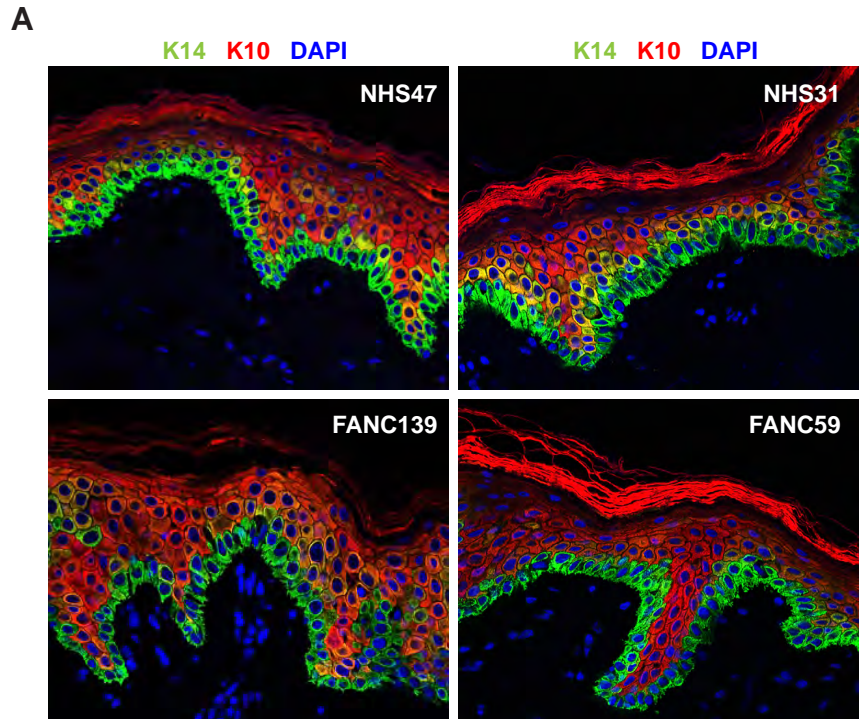
Relative expression of the  $\Delta$ Np63 basal cell marker in ESPCs at passage 1, normalized to the expression in normal human skin keratinocytes (NHSK); n=2. Error bars represent SEM. **(B)** Cell cycle analysis of ESPCs in the presence or absence of 100 ng/mL DOX reveals no changes in proliferation in monolayer cells, as measured by EdU incorporation; n=2, one representative example is shown.

Figure S3 Related to Figure 3



**Figure S3 Related to Figure 3: FA pathway loss stimulates proliferation in cutaneous and oral epidermis.** Quantification of EdU+ cells on 3D engineered epidermis derived from control NTsh or FANCD2 depleted NIKS (**A**) or NOKS (**B**); n=1. Bottom right insets confirm decreased protein expression of FANCD2 in the FANCD2-depleted NIKS (**A**) or NOKS (**B**). Error bars represent SEM. Scale, 50  $\mu$ m.

Figure S4 Related to Figure 4



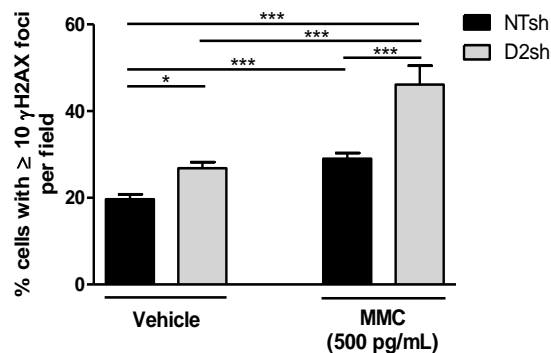
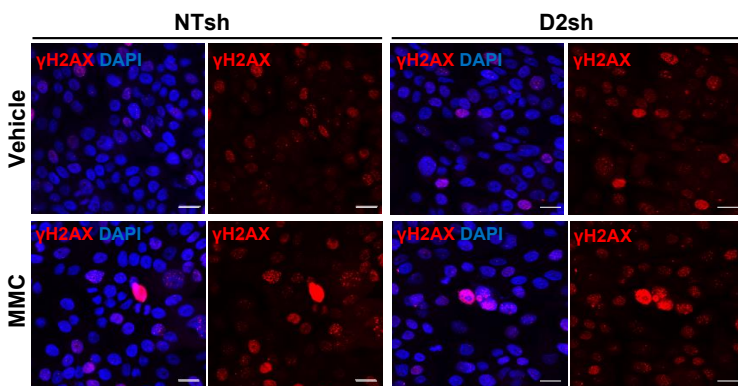
**Figure S4 Related to Figure 4: FA pathway loss does not impair epidermal differentiation.**

(A) Sections from skin punch biopsies of 2 controls (NHS) and 2 individuals with FA (FANC) were stained for the differentiation markers K14 and K10, and could not be distinguished from each other based on examination by a pathologist blinded to FA status. (B) Skin-punch biopsies from 2 affected individuals and 2 gender-matched controls, showing no differences in the overall architecture of FA epidermis. Scale bar, 100  $\mu$ m.

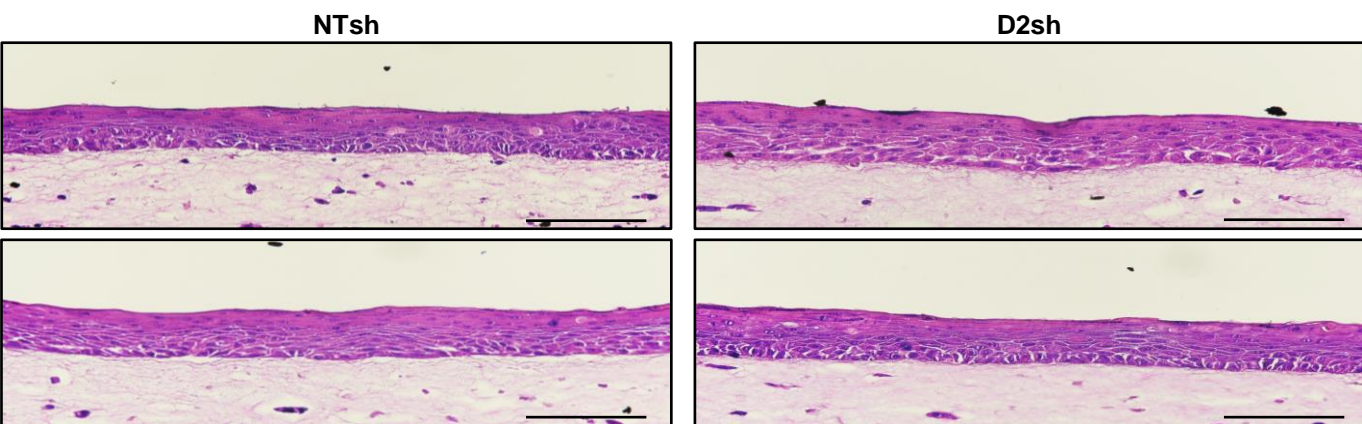


Figure S5 Related to Figure 5

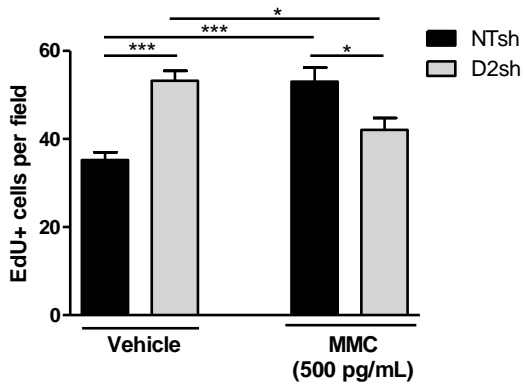
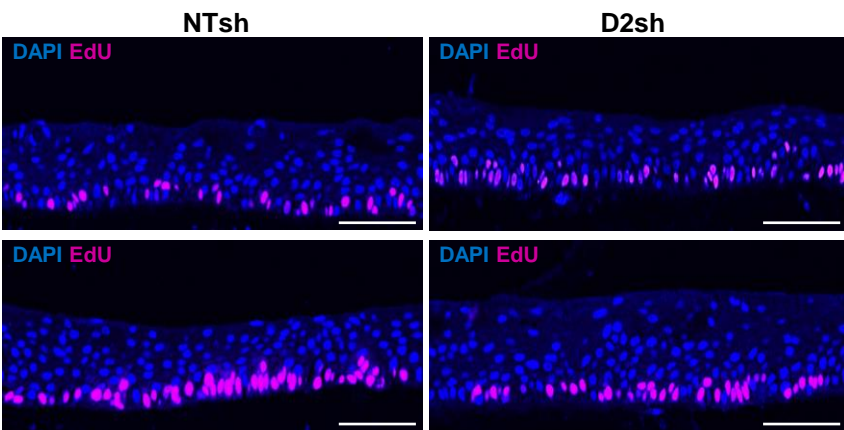
**A**



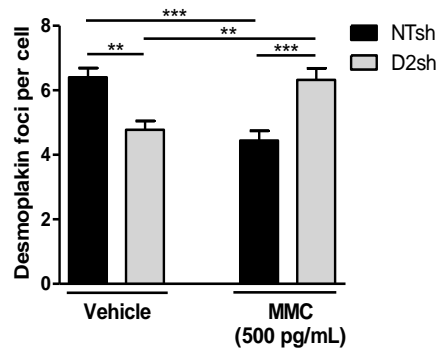
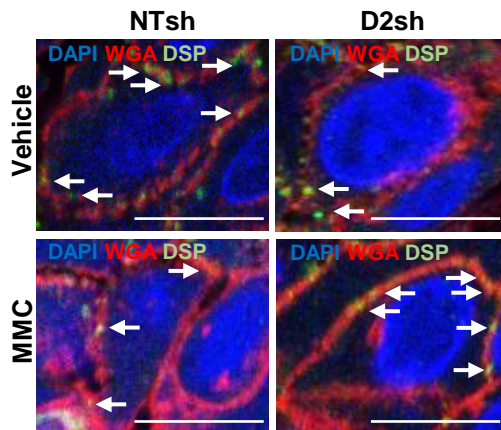
**B**



**C**



**D**



**Figure S5 Related to Figure 5: DNA damage mimics the functional defects of FA-EORs in normal epidermis.** (A) NTsh and FANCD2sh (D2sh) NIKS were pretreated with vehicle or a nontoxic dose of MMC (500 pg/mL) and stained for  $\gamma$ H2AX. Representative images and quantification of cells with  $\geq 10$   $\gamma$ H2AX foci are shown. At least 170  $\gamma$ H2AX+ cells were counted per condition. \* $P < 0.05$  and \*\*\* $P < 0.001$  (1-way ANOVA, n=1 biological replicate, n=4-10 fields). Error bars represent SEM. Scale bar, 20  $\mu$ m. (B) Representative images of H&E staining showing normal overall architecture on 3D engineered epidermis derived from NTsh and D2sh NIKS continuously treated with vehicle or MMC (500 pg/mL). Scale bar, 100  $\mu$ m. (C) Quantification of EdU+ cells in 3D engineered epidermis derived from NTsh and D2sh NIKS continuously treated with vehicle or MMC (500 pg/mL). \* $P < 0.05$  and \*\*\* $P < 0.001$  (1-way ANOVA, n=1 biological replicate, n=3 technical replicates). Error bars represent SEM. Scale bar, 100  $\mu$ m. (D) Sections from NTsh and D2sh NIKS continuously treated with vehicle or MMC (500 pg/mL) were stained for DSP 1/2 and the cell membrane marker wheat germ agglutinin (WGA). Images and quantification of DSP foci per cell are shown. Arrows: DSP foci on membrane. \*\* $P < 0.01$  and \*\*\* $P < 0.001$  (1-way ANOVA, n=1 biological replicate, n=2 technical replicates). Error bars represent SEM. Scale bar, 10  $\mu$ m.

**Table S1 Related to Table 1 and Figure S1: FA somatic mutations in sporadic cancers.**

Gene	Mutations in AML	Mutations in HN SCC	Mutations in Skin SCC
FANCA	-	K143N, D533N, S394C	S645F, S113L, R1243K, S1149F, A1227T, K847N, P128S
FANCB	-	V301L, I76M, I418Nfs*18	P610L
FANCC	E43G	C10Y, E259*	P360S
FANCD1/BRCA2	-	E2903K, V1605D, G500V, E1571K, I2105V, P606L, K3315N, E3342K, R2625*, X211_splice	A2595G, P2477L, P3292S, P3292L, P2153L, Q2160H, R2787H, P655R, C1960G, P2334S, L3011F, P555S, W2586*
FANCD2	-	Q1010R, S250T, X426_splice	F533C, S603C, S1145*
FANCE	-	-	A126V
FANCF	-	-	D105G
FANCG	-	S33C, D604N	S425L
FANCI	S1011T	I741M, D802N, Q74E	I877L, F763=
FANCJ/BRIP1	-	S1115F, G750V, Q867L, W863L, T411A, D280N	E81*
FANCL	-	M305V	P249L, P249S
FANCM	G535S	R185T, S832C, R92P, V1336Lfs*2	P962S, S1466L
FANCN/PALB2	-	D586N, N444S, S489F, C828F	L482F, R501K
FANCO/RAD51C	-	P18R, 120Qfs*21	-
FANCP/SLX4	-	S1608L, E1780K, L193F, P1477S, S659L	-
FANCQ/ERCC4	-	R138K, E694Q, D287N, P550S	S283F, P550S, E549K
FANCR/RAD51	-	S296L	-
FANCS/BRCA1	-	D853N, G1492R, E230Q, D1505N, R664G, R1645M, S1241F, H816Y, X101_splice	P985L, L1439F, P1192L, P1192S
FANCT/UBE2T	-	-	-
FANCU/XRCC2	-	-	-
FANCV/MAD2L2	-	Y119F, E106*	-
FANCW/RFWD3	-	Q19L, E709K, A17V	-

**Table S1 Related to Table 1 and Figure S1: FA somatic mutations in sporadic cancers.** List of FA non-synonymous mutations in AML, HN SCC, and skin SCC obtained from TCGA data.

**Table S2 Related to Figure 4: Characteristics of skin biopsy donors used for pigment incontinence studies.**

<b>Donor</b>	<b>Gender</b>	<b>Age (years)</b>	<b>Race</b>
NHS66	M	26	Not Reported
NHS64	F	37	Caucasian
NHS47	M	20	African-American
NHS42	F	28	Caucasian
NHS39	F	24	Not Reported
NHS38	M	20	Caucasian
NHS33	F	28	Caucasian
NHS31	F	24	Not Reported
NHS30	M	21	Not Reported
FANC144	F	16	Caucasian
FANC139	F	23	Caucasian
FANC92	M	13	Caucasian
FANC89	F	20	Caucasian
FANC65	M	4	Caucasian
FANC60	F	19	Caucasian
FANC59	F	18	Caucasian
FANC51	M	19	African-American
FANC40	F	3	Caucasian

**Table S2 Related to Figure 4: Characteristics of skin biopsy donors used for pigment incontinence studies.** Table showing the characteristics for individuals with FA (FANC) and gender-matched control (NHS) donors that provided skin-punch biopsies for the pigment incontinence experiments. Abbreviations: NHS= gender-matched normal human skin donor; FANC= clinically diagnosed FA donor; M=Male; F=Female.

**Table S3 Related to Figure 4: Characteristics of skin biopsy donors used for EM analyses.**

<b>Donor</b>	<b>Gender</b>	<b>Age (years)</b>
NHS57	F	21
NHS58	M	23
NHS59	F	33
NHS60	M	20
NHS62	F	30
NHS63	M	35
FANC116	F	34
FANC131	M	5
FANC132	M	6
FANC134	M	17
FANC135	M	2
FANC136	M	3
FANC138	F	15
FANC139	F	23
FANC140	F	25

**Table S3 Related to Figure 4: Characteristics of skin biopsy donors used for EM analyses.**

Table showing the characteristics for individuals with FA (FANC) and gender-matched control (NHS) donors that provided skin-punch biopsies for EM analyses. Abbreviations: NHS= gender-matched normal human skin donor; FANC= clinically diagnosed FA donor; M=Male; F=Female.



**Table S4: List of antibodies used for IF and WB analyses. Related to STAR Methods.**

<b>Antibody</b>	<b>Company</b>	<b>Cat. Number</b>	<b>Dilution</b>
Rabbit anti-Krt14	BioLegend	905301	1:2,000
Mouse anti-phospho-Histone H2A.X Ser139	Millipore	05-636	1:400
Mouse anti-cytokeratin 10	Abcam	ab9025	1:250
Rabbit anti-FANCD2	Novus	NB100-182	1:400 IF 1:2,000 WB
Mouse anti-Vinculin	Sigma-Aldrich	V9131	1:5,000
Rabbit anti-FANCA	Cascade BioScience	ABP-6201	1:1,000
Wheat Germ Agglutinin, tetramethylrhodamine conjugate	Thermo Fisher Scientific	W849	1:10
Mouse anti-Desmoplakin 1/2, clone DP447, supernatant	Progen Biotechnik	651155	1:10
Donkey anti-rabbit IgG HRP-conjugated	GE Healthcare	NA934	1:5,000
Sheep anti-mouse IgG HRP-conjugated	GE Healthcare	NA931	1:2,500
AlexaFluor Goat anti-rabbit 488	Thermo Fisher Scientific	A-11008	1:500
AlexaFluor Goat anti-mouse 568	Thermo Fisher Scientific	A-11004	1:500

**Table S5: List of primers used for RT-qPCR analyses. Related to STAR Methods.**

<b>Gene</b>	<b>Specie</b>	<b>Strand</b>	<b>Sequence</b>
<i>ΔNp63</i>	Human	Forward	ATTGTAAGGGTCTCGGGGTGGG
		Reverse	GAGTCTGGGCATTGTTTTCCAGGT
<i>GAPDH</i>	Human	Forward	GGTCTCCTCTGACTTCAACA
		Reverse	ATACCAGGAAATGAGCTTGA
<i>NOTCH1</i>	Human	Forward	TGAATGGCGGGAAGTGTGAAG
		Reverse	CATTGTCCAGGGGTGTCAGG
<i>VWCE</i>	Human	Forward	GTGTAGATGTAAACGAGTGTCCG
		Reverse	GTCGGCATGTGCATAGGAAG
<i>PLXNB3</i>	Human	Forward	TACGGGTGTCGGACAATGTG
		Reverse	CATCTGCTTGTAGCGTGGGA
<i>LGALS4</i>	Human	Forward	CGACGCTGCCTTACTACCAG
		Reverse	CCAACCACAAAGTTCACGAAGA
<i>CLDN9</i>	Human	Forward	AGACACACCCTCTGAGTCACCTAGG
		Reverse	ACGATGCTGTTGCCGATGAAG
<i>PCDHB6</i>	Human	Forward	AACGGCCTTCAGAGGTACAC
		Reverse	CCAACGGTTTGTCTAGCACCA