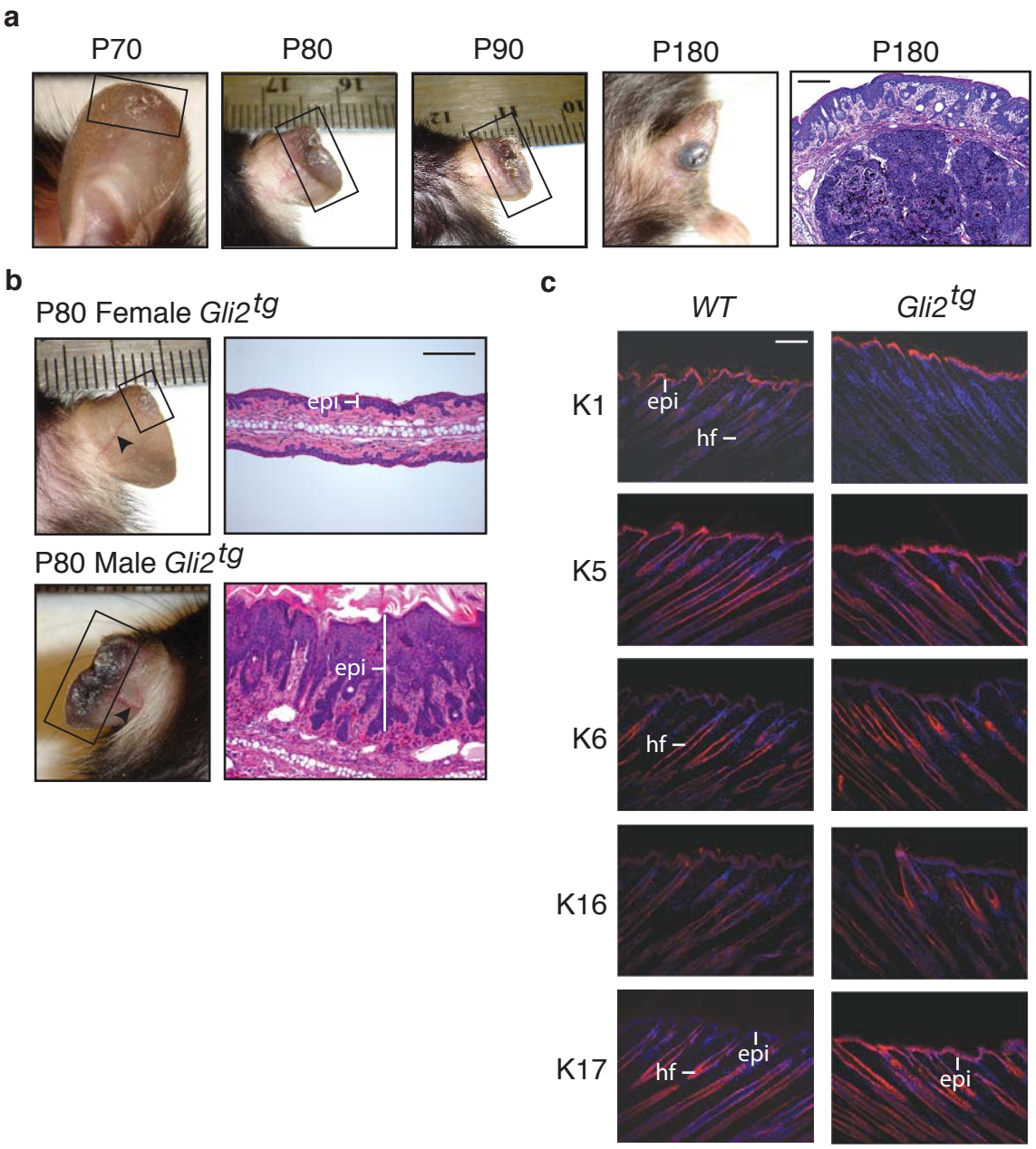
Keratin 17 promotes epithelial proliferation and tumor growth by polarizing the immune response in skin

Daryle DePianto, Michelle Kerns, Andrzej A. Dlugosz and Pierre A. Coulombe

Supplemental Figure 1
DePianto et al.



Characterization of the *Gli2tg* mouse model of basal cell carcinoma.
(a) Macroscopic presentation of developing ear lesions at ages P70, P80, and P90 in male *Gli2tg* mice. **(b)** Gender bias within the *Gli2tg* mouse model. Female mice have a delayed onset of ear lesions compared to males (compare boxed areas). Arrows denote blood vessels, which get progressively larger as tumorigenesis proceeds. **(c)** Keratin expression in back skin of P14 wild type (*WT*) and *Gli2tg* mice. The antigen being detected is given at left. K17 is robustly induced in interfollicular epidermis of *Gli2tg* backskin, and is the only keratin epitope, among those surveyed, showing an altered regulation at P14. Epi, epidermis; hf, hair follicle. Scale bars: 25μm.

Supplemental Figure 2
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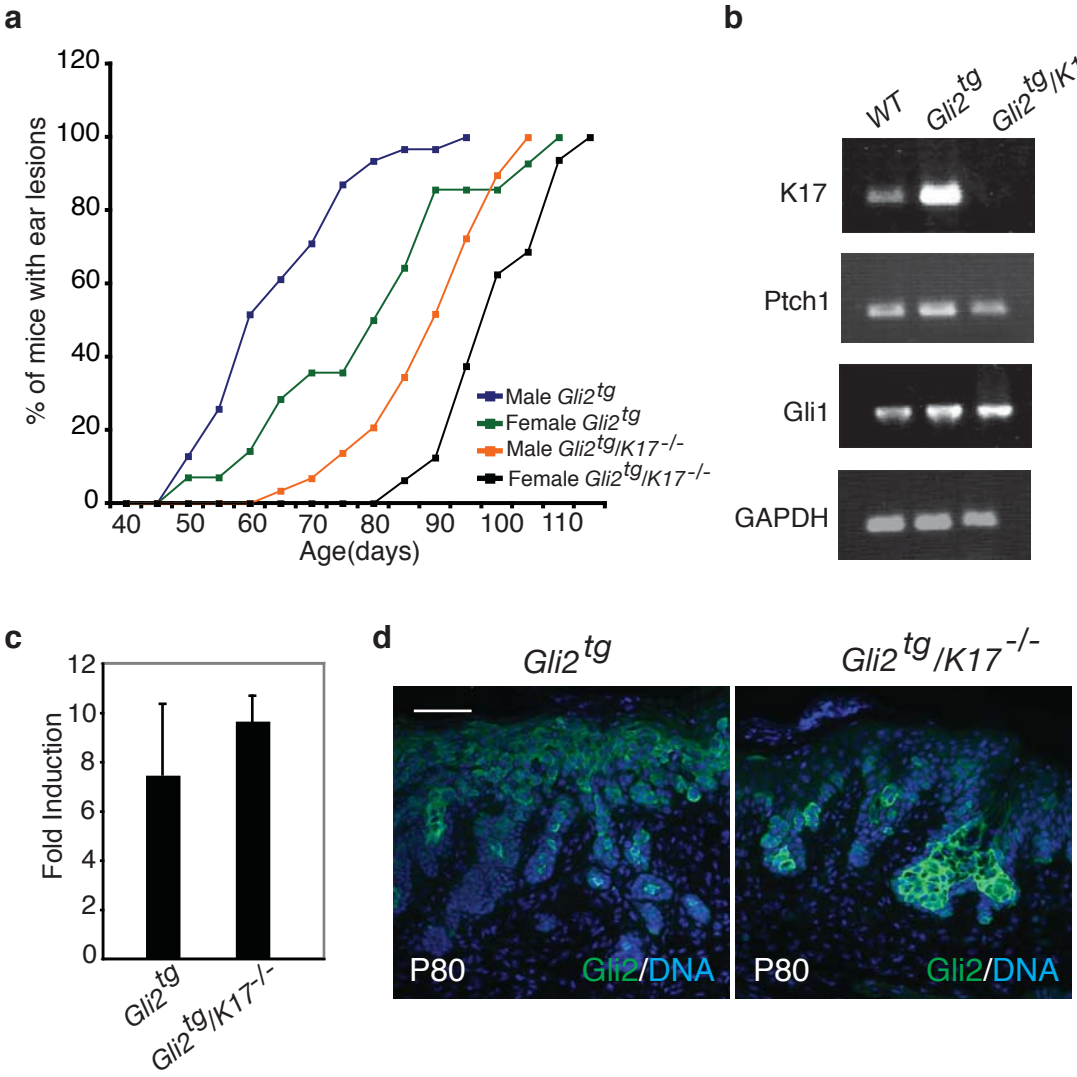
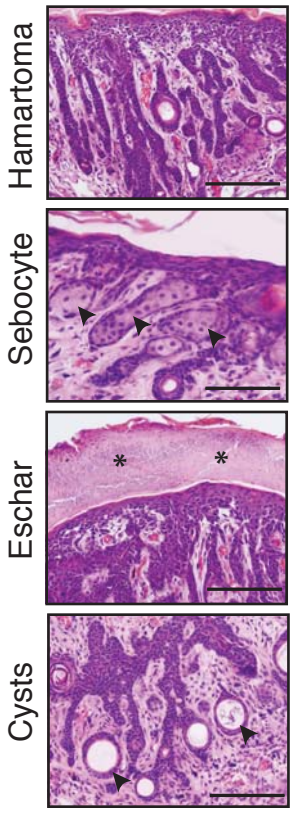


Analysis of tumor onset and associated histological attributes in *Gli2tg* and *Gli2tg/K17-/-* mice. **(a)** Tumor kinetics, related as the cumulative percentage of mice showing marked ear lesions in male and female *Gli2tg* and *Gli2tg/K17-/-* mice during the P40-P120 time frame. This representation complements the histogram shown in Figure 1B. **(b)** Semi-quantitative RT-PCR for targets of the Hedgehog signaling pathway (Patched 1, Gli1, and K17) in P80 ear tissue of male mice. GAPDH is used as an internal reference. **(c)** Relative luciferase activity of a Gli2 responsive reporter construct transfected (8xK17bs-luc; see Methods) into keratinocytes isolated from newborn *Gli2tg* and *Gli2tg/K17-/-* mice, and cultured. Data was normalized to fold induction over the pGL3-Basic vector (again, see Methods). Values reported as mean ± s.e.m. ($p < 0.05$). **(d)** Gli2 immunostainings of P80 *Gli2tg* and *Gli2tg/K17-/-* ear sections. Scale bar: 25µm.

Supplemental Figure 3
DePianto et al.

a



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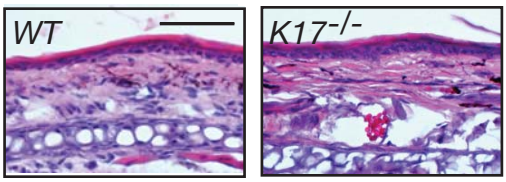
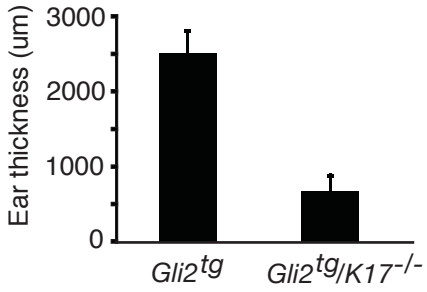


c

Genotype	Gender	Age	Hamartoma	Eschar	Sebocyte	Cyst
Gli2^{tg}	M	p80	2.00	2.16	1.33	1.00
Gli2^{tg}/K17$^{-/-}$	M	p80	1.00	0.75	0.00	0.00
Gli2^{tg}	M	p90	2.50	2.67	1.50	0.83
Gli2^{tg}/K17$^{-/-}$	M	p90	1.00	1.00	0.50	0.00
Gli2^{tg}	M	p100	2.80	2.80	1.30	1.16
Gli2^{tg}/K17$^{-/-}$	M	p100	0.75	0.50	0.00	0.25
Gli2^{tg}	M	p125	2.50	3.00	1.50	2.00
Gli2^{tg}/K17$^{-/-}$	M	p125	1.50	0.50	1.00	0.50

d



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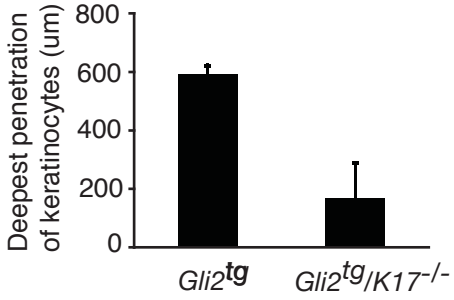

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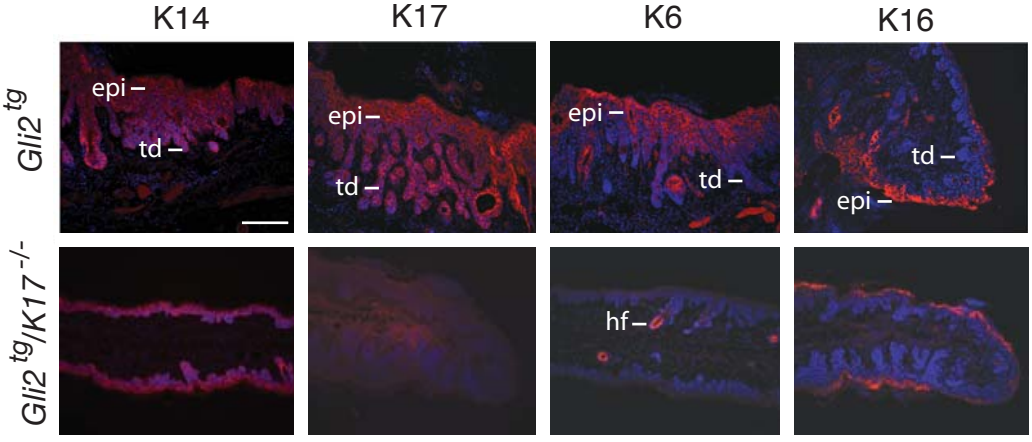

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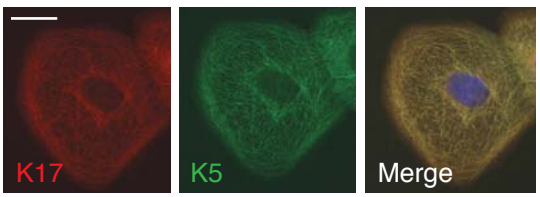

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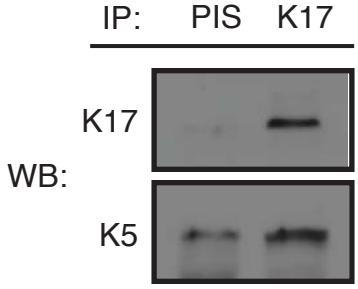

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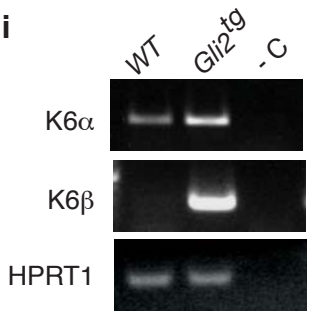
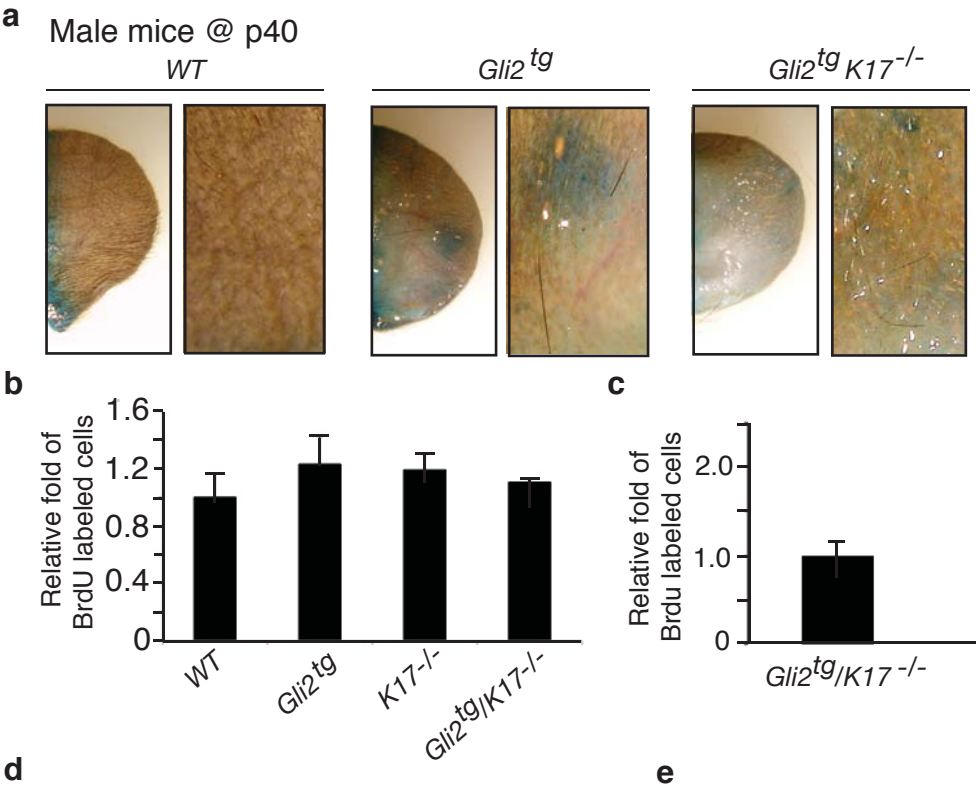


Supplemental Figure 3
DePianto et al.

Key histological features associated with development of ear lesion in *Gli2tg* mice.
(a) Hamartoma, eschar (see asterisks), sebocytes (arrows), and cyst (arrows) lesions are individually featured. **(b)** Hematoxylin-eosin stained ear sections of P80 wildtype and *K17-/-* mice, depicting the normal histology that prevails in the absence of K17. Scale bar: 40μm. **(c)** Quantification of key histological features in *Gli2tg* and *Gli2tg/K17-/-* ear tissue at various ages. An experienced observer of skin tumors (author A.A.D.) scored slides in a blind fashion using a semi-quantitative scale for the features illustrated in a. Scale bars: hamartoma, eschar, cyst (50μm), sebocyte (25μm **(d)** Thickness of whole ear tissue, in histological cross-sections, in P80 male *Gli2tg* and *Gli2tg/K17-/-* mice.
(e) Measurement of deepest keratinocyte penetration into underlying dermal tissue in P80 male *Gli2tg* and *Gli2tg/K17-/-* mice. For C, D values are reported as mean ± s.e.m. The difference between the two groups is statistically significant, $p < 0.05$. **(f)** Keratin expression in P80 male *Gli2tg* and *Gli2tg/K17-/-* ear tissue. The antigen being localized is identified on top. Scale bar, 50μm.
(g) Co-localization of K5 and K14 proteins in *Gli2tg* primary keratinocytes visualized via indirect immunofluorescence. Scale bar: 20μm. **(h)** Co-immunoprecipitation of K5 and K17 from tissue extracts of *Gli2tg* ear lesions. Pre-immune sera (PIS) was used as a control. **(i)** Semi-quantitative RT-PCR for K6 isoform α and β mRNAs expressed in ear lesions of *Gli2tg* mice. HPRT1 is used as an internal reference.

Supplemental Figure 4
DePianto et al.



d

Cytok/Chemok (Th1)	Fold Change	P-Value
Cxcl11	-36.96	0.050
Cxcl5	-3.20	0.050
Cxcl10	-2.56	0.040
Ccl9	-2.13	0.195
Ccl12	-2.02	0.111
Cxcl9	-1.41	0.421
IL1β	-1.50	0.358
Ccr1	1.07	0.741

Cytok/Chemok (Th2)	Fold Change	P-Value
IL20	1.35	0.353
IL4	-1.43	0.952
IL13	-1.55	0.139
Ccl24	-1.55	0.139
Ccl17	-1.70	0.247

e

Cytok/Chemok (Th1)	Fold Change	P-Value
Cxcl11	-1.17	0.668
Cxcl5	3.00	0.001
Cxcl10	-1.17	0.668
Ccl9	-1.10	0.655
Ccl12	-1.10	0.111
Cxcl9	96.92	0.001
IL1β	-1.28	0.319
Ccr1	-1.17	0.668

Cytok/Chemok (Th2)	Fold Change	P-Value
IL20	1.16	0.318
IL4	-1.10	0.538
IL13	-1.26	0.824
Ccl24	-1.12	0.951
Ccl17	-1.17	0.688

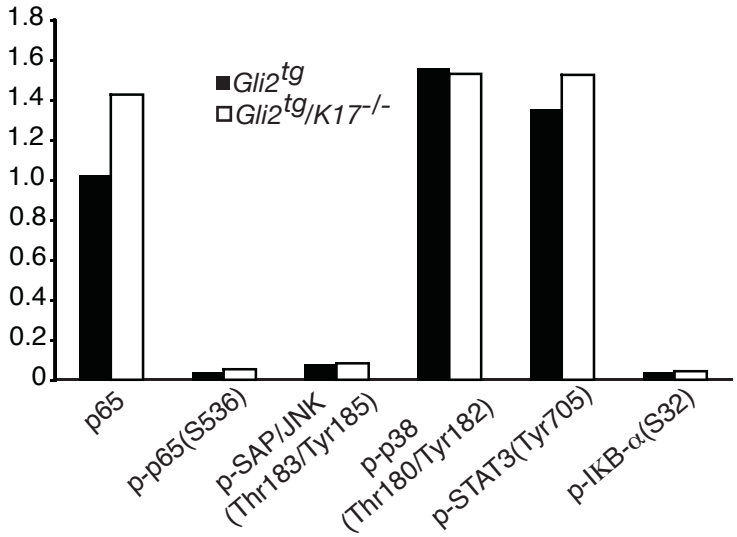
Examination of inflammation and its effects in ear skin tissue and skin keratinocyte cultures. **(a)** Integrity of skin barrier in wildtype, *Gli2tg*, and *Gli2tg/K17-/-* mice. **(b)** Mitotic activity of wildtype, *K17-/-*, *Gli2tg*, and *Gli2tg/K17-/-* keratinocytes seeded in primary culture after isolation from newborn mice as gauged by BrdU incorporation. Values are reported as mean ± s.e.m. **(c)** Quantification of BrdU incorporation in *Gli2tg* and *Gli2tg/K17-/-* keratinocytes in primary culture after a 12 hour treatment with 2μM TPA. Values are reported as mean ± s.e.m. **(d)** qRT-PCR quantification of mRNA expression levels of various chemokines and cytokines from *Gli2tg/K17-/-* versus *Gli2tg* primary cell cultures treated for 12 hours with 2μM TPA. (e) qRT-PCR quantification of mRNA expression levels of various chemokines and cytokines from *Gli2tg/K17-/-* transfected with a K17 expression construct compared to mock-transfected *Gli2tg/K17-/-* keratinocytes in primary culture, after a 12 hour treatment with 2μM TPA. The data reported correspond to the average of three experiments.

Supplemental Figure 5
DePianto et al.

a

NF-κB Signaling	Fold Change	P-Value
Traf2	-3.08	0.0057
Irf1	-2.28	0.0031
Tnfrsf1b	-1.96	0.0043
Tlr2	-1.82	0.0165
Rela	-1.75	0.0306
Relb	-1.73	0.0118
Jun	-1.69	0.0318
Atf1	-1.37	0.0388
Ripk2	-1.23	0.5151
Akt1	-1.21	0.6387
Ikbkb	-1.21	0.1923
Tradd	1.02	0.9134
Icam1	1.40	0.0280

b



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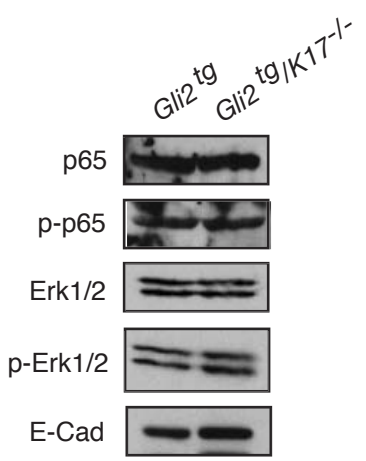

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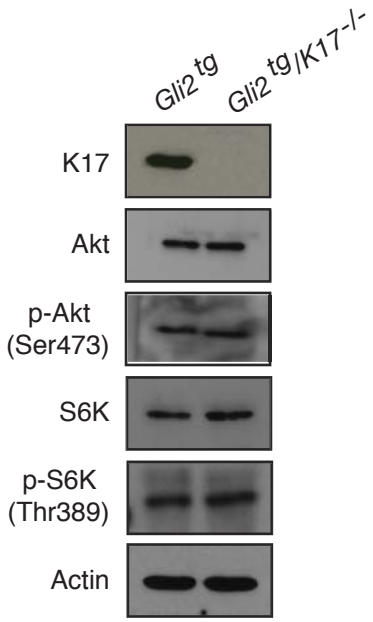

Status of various signaling pathways in cultured skin keratinocytes and in P80 male ear tissue. **(a)** NFκB signaling pathway activity in TPA-treated primary cultures of newborn *Gli2tg/K17-/-* versus *Gli2tg* skin keratinocytes examined via qPCR array. **(b)** Activation of inflammation and stress-related pathways were evaluated via ELISA assays on whole ear tissue. Data (average of three experiments) is normalized to p65 levels in *Gli2tg* mice. **(c)** Total and phosphorylated levels of p65 and Erk1/2 in whole ear skin tissue (P80 mice). E-cadherin is a loading control for epithelial cells. **(d)** Total and phosphorylated levels of Akt and S6K in primary keratinocyte cultures. Actin is used as a loading control.

Supplementary Table 1 - Oligonucleotide primer pairs and reaction conditions used for the PCR-based detection of SELECT genes or mRNA transcripts

DePianto et al.

Gene/Transcript	Primer Sequence (5'-3')	PCR Parameters
b-Globin intron	Forward: TGCATATAAATTCTGGCTGGCG Reverse: GCATGAACATGGTTAGCAGAGGG	94°C 40sec 58°C 40sec 72°C 40sec 27 cycles
K17 wt allele	Forward: GGCCAGGTGGGCGGCGAAATCAAC Reverse: GAGCCTGGACCCTTCCCGAAGTCAG	94°C 40sec 62°C 40sec 72°C 40sec 35 cycles
K17 null allele	Forward: GGCCAGGTGGGCGGCGAAATCAAC Reverse: CGCAGCGCATCGCCTTCTATCGCCT	94°C 40sec 62°C 40sec 72°C 40sec 35 cycles
K5/Gli2^{tg}	Forward: ACAAGGACGACGATGACAAG Reverse: AGTCCCCTCTCTTTCAGATG	94°C 40sec 56°C 60sec 72°C 60sec 30 cycles
GAPDH	Forward: AAATGGTGAAGGTCGGTGT Reverse: ACTCCACGACATACTCAGCAC	94°C 40sec 58°C 50sec 72°C 50sec 30 cycles
TSLP	Forward: TCAGGAGCCTCTTCATCCTGC Reverse: TGTTTTGGACTTCTTGTGCCA	94°C 30sec 57°C 30sec 72°C 45sec 34 cycles
mDefb14	Forward: CATTATCTGCTATTTGTATTCC Reverse: CTTTCGGCAGCATTTTCGACC	94°C 40sec 54°C 35sec 72°C 45sec 32 cycles
S100A8	Forward: GAAAATGGTCACTACTGAGTGTCCT Reverse: GACTTTATTCTGTAGACATATCCAG	94°C 40sec 65°C 30sec 72°C 45sec 33 cycles

HPRT1	Forward: CACAGGACTAGAACACCTGC Reverse: CTGGTGAAAAGGACCTCT	94°C 40sec 50°C 30sec 72°C 30sec 32 cycles
mGli1	Forward GTCGGAAGTCCTATTCACGC Reverse CAGTCTGCTCTCTTCCCTGC	95°C 50sec 58°C 30sec 72°C 60sec 32 cycles
K17 mRNA	Forward: GGGAGCAGCAGAACCAGGAGTAC Reverse: GTCTCAAGCATAGGAATGCTGGGG	94°C 40sec 60°C 30sec 72°C 40sec 30 cycles
Patched-1	Forward: CAGACCACGGTGTCTGGCATC Reverse: GCAGAAGCCGTCACAGTGGTG	94°C 40sec 62°C 45sec 72°C 45sec 30 cycles