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Supplemental information

Basal cell carcinomas acquire secondary mutations to overcome dormancy and progress from microscopic to macroscopic disease

Kenneth G. Trieu, Shih-Ying Tsai, Markus Eberl, Virginia Ju, Noah C. Ford, Owen J. Doane, Jamie K. Peterson, Natalia A. Veniaminova, Marina Grachtchouk, Paul W. Harms, Fredrik J. Swartling, Andrzej A. Dlugosz, and Sunny Y. Wong

Trieu_Fig S1

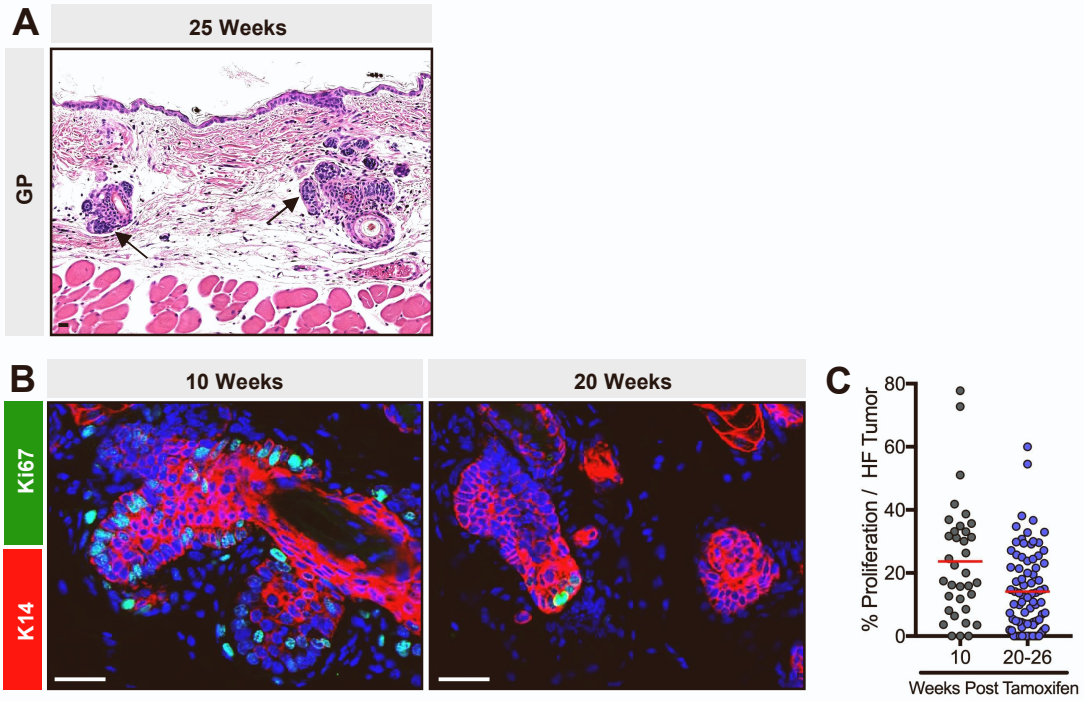

Figure S1. Tumors arising in GP and SmoM2 mice fail to progress. Related to Figure 2.
A. Histology of microscopic hair follicle (HF)-associated GP tumors (arrows), 25 weeks post-TAM. **B.** HF-associated SmoM2 tumors exhibit reduced proliferation over time, as assessed by Ki67 (green). **C.** Quantitation of proliferation in HF-associated SmoM2 tumors. Significance for beeswarm plot was calculated using a linear mixed model ($p = 0.02$). Scale bar, 50 μm.

Trieu_Fig S2

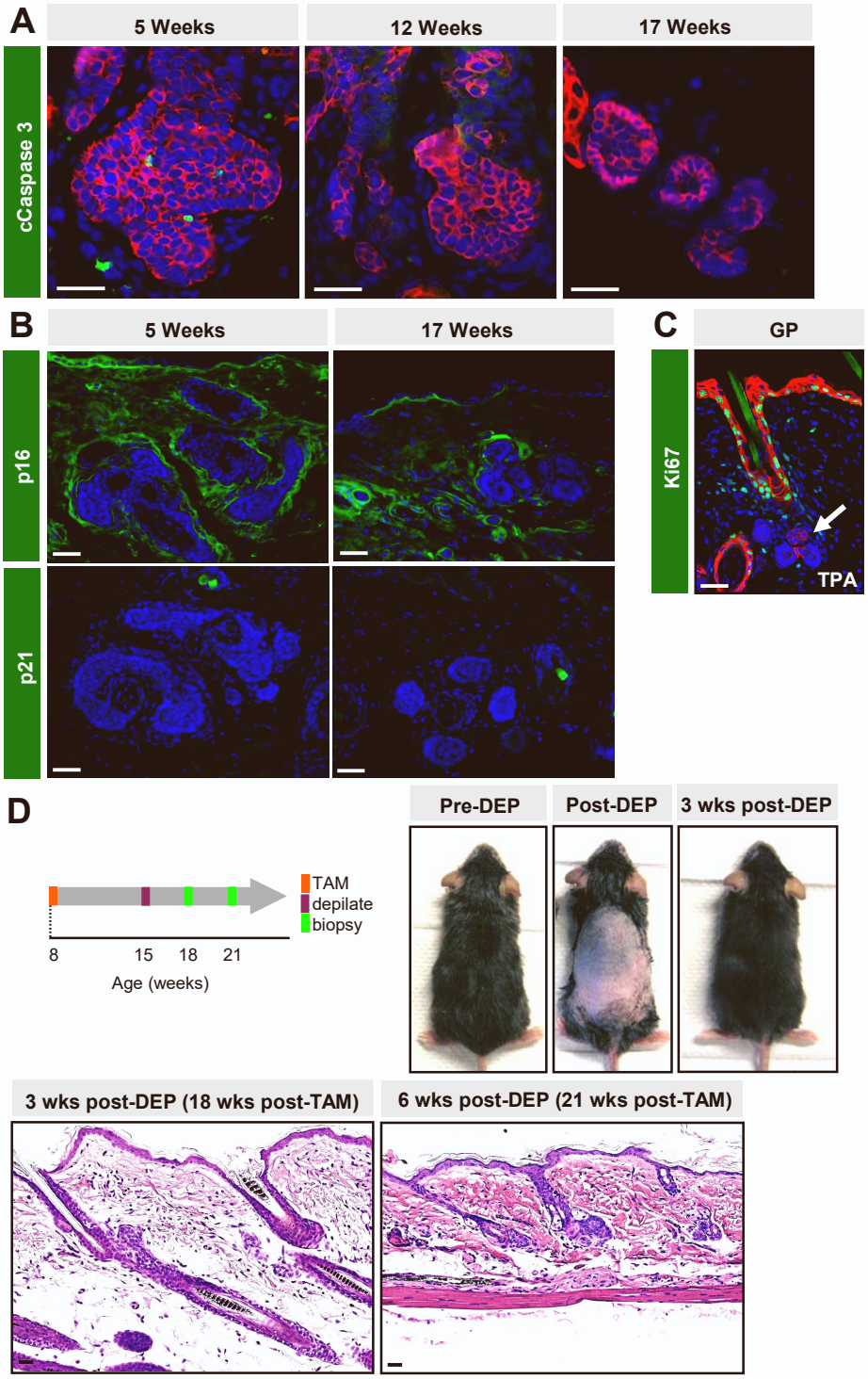

Figure S2. Dormant GP tumors do not progress in response to phorbol ester treatment or depilation. Related to Figure 2. A. Staining for cleaved Caspase 3 (green) in GP tumors, 5-17 weeks post-TAM. Red, K14. **B.** Lack of staining for senescence markers p16 (green, top) and p21 (green, bottom) in GP tumors, 5 and 17 weeks post-TAM. **C.** TPA treatment does not induce proliferation, as assessed by Ki67 (green) in GP tumors (arrow). **D.** Schematic for depilation and skin biopsy in GP mice. Mice were also biopsied at the end of the experiment. Right, photos showing dorsal skin from GP mice, pre- and post-depilation (DEP). Bottom photos, histology of GP mice, 3-6 weeks post-depilation. Scale bar, 50 μm.

Trieu_Fig S3

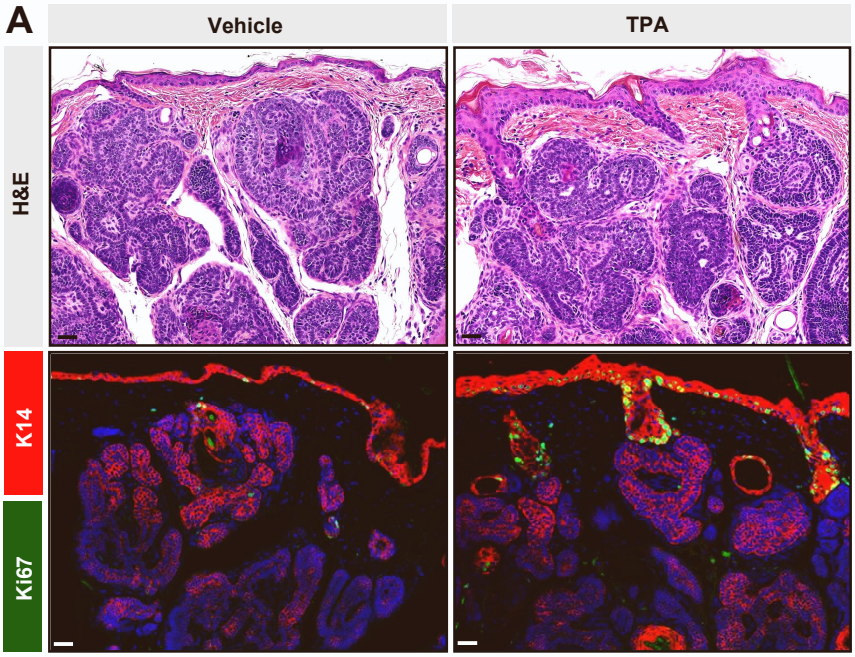

Figure S3. Phorbol ester treatment does not restore proliferation in GPN1 tumors. Related to Figure 3. A. Top, histology of vehicle- and TPA-treated GPN1-KO tumors. Bottom, proliferation, as assessed by Ki67 (green), is not restored in GPN1 tumors treated with TPA, even though elevated proliferation is seen in the interfollicular epidermis and upper hair follicle. Scale bar, 50 μm.

Trieu_Fig S4

A

		Tumor Code	CNV	SNV	InDel
GPP53	9854-C1	C1_9854	116	10	0
	5452-C1	A2_1	294	5	1
	5365-C1	A5_1	182	10	0
	5364-C2	A4_2	304	4	0
	5558-C2	A58_2	512	3	0
	5580-C1	A80_C1	191	9	2
	5579-C1*	A79_1	151	8	2
	5364-C3	A4_3	503	6	1
	5365-C2	A5_2	335	6	0
	5452-C2	A2_2	510	7	0
	5398-C1	A8_1	554	5	3
	5580-C2	A80_C2	77	5	3
	5558-C1	A58_1	870	3	0
	5420-C1	A20_1	239	3	0
	9883T	A83_1	72	5	0
	9851*	C1_9851	37	3	0
GPN1	5274-C1	A74_1	135	10	0
	666	C1_666	36	6	3
	9577	C1_9577	76	7	0
	5432-C3	A32_3	55	4	0
	5435-C1	A35_1	39	7	1

B

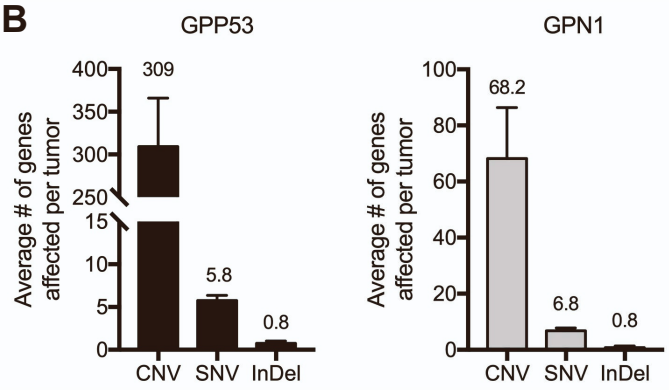

Figure S4. Summary of genomic alterations in GPP53 and GPN1 macroscopic tumors. Related to Figure 5. A. Table indicating the number of genes affected by different classes of somatic mutations for each tumor analyzed by WES: copy number variation (CNV), single nucleotide variation (SNV) and insertion/deletion (InDel). Asterisk, GPP53-Het tumor. **B.** Average number of genes affected by CNVs, SNVs and InDels in GPP53 and GPN1 macroscopic tumors.

Trieu_Fig S5

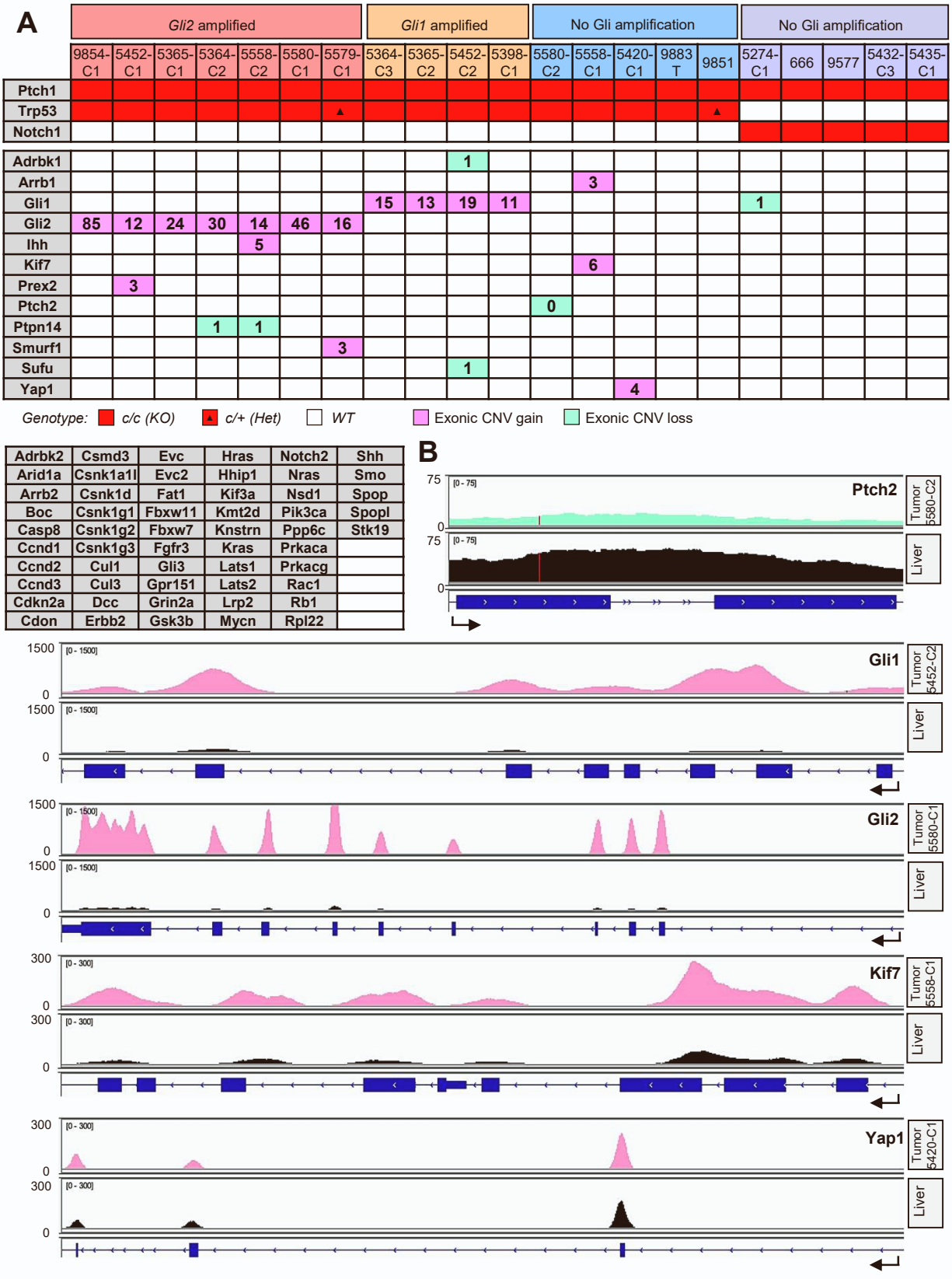
A
Gli2 amplified
Gli1 amplified
No Gli amplification
No Gli amplification
9854-C1
5452-C1
5365-C1
5364-C2
5558-C2
5580-C1
5579-C1
5364-C3
5365-C2
5452-C2
5398-C1
5580-C2
5558-C1
5420-C1
9883 T
9851
5274-C1
666
9577
5432-C3
5435-C1
Ptch1
Trp53
Notch1
Adrbk1
Arrb1
Gli1
Gli2
Ihh
Kif7
Prex2
Ptch2
Ptpn14
Smurf1
Sufu
Yap1
Genotype: c/c (KO) c/+ (Het) WT Exonic CNV gain Exonic CNV loss
Adrbk2 Csmd3 Evc Hras Notch2 Shh
Arid1a Csnk1a1l Evc2 Hhip1 Nras Smo
Arrb2 Csnk1d Fat1 Kif3a Nsd1 Spop
Boc Csnk1g1 Fbxw11 Kmt2d Pik3ca Spopl
Casp8 Csnk1g2 Fbxw7 Knstrn Ppp6c Stk19
Ccnd1 Csnk1g3 Fgfr3 Kras Prkaca
Ccnd2 Cul1 Gli3 Lats1 Prkacg
Ccnd3 Cul3 Gpr151 Lats2 Rac1
Cdkn2a Dcc Grin2a Lrp2 Rb1
Cdon Erbb2 Gsk3b Mycn Rpl22
B
Ptch2
Gli1
Gli2
Kif7
Yap1
Tumor 5580-C2
Liver
Tumor 5452-C2
Tumor 5580-C1
Tumor 5558-C1
Tumor 5420-C1

Figure S5. Summary of somatic CNVs in macroscopic tumors. See also Figure 5. A. List of 70 commonly mutated genes in BCC (gray), and table showing CNVs affecting these genes in 16 GPP53 and 5 GPN1 macroscopic tumors. Bottom table is the remaining list of genes that were not found to be mutated in any of the 21 tumors. **B.** Visualization of WES traces for *Ptch2* loss (tumor 5580-C2), *Gli1* amplification (tumor 5452-C2), *Gli2* amplification (tumor 5580-C1), *Kif7* amplification (tumor 5558-C1) and *Yap1* amplification (tumor 5420-C1). Each tumor (pink or teal trace) is compared against its matched normal (liver, black trace).

Trieu_Fig S6

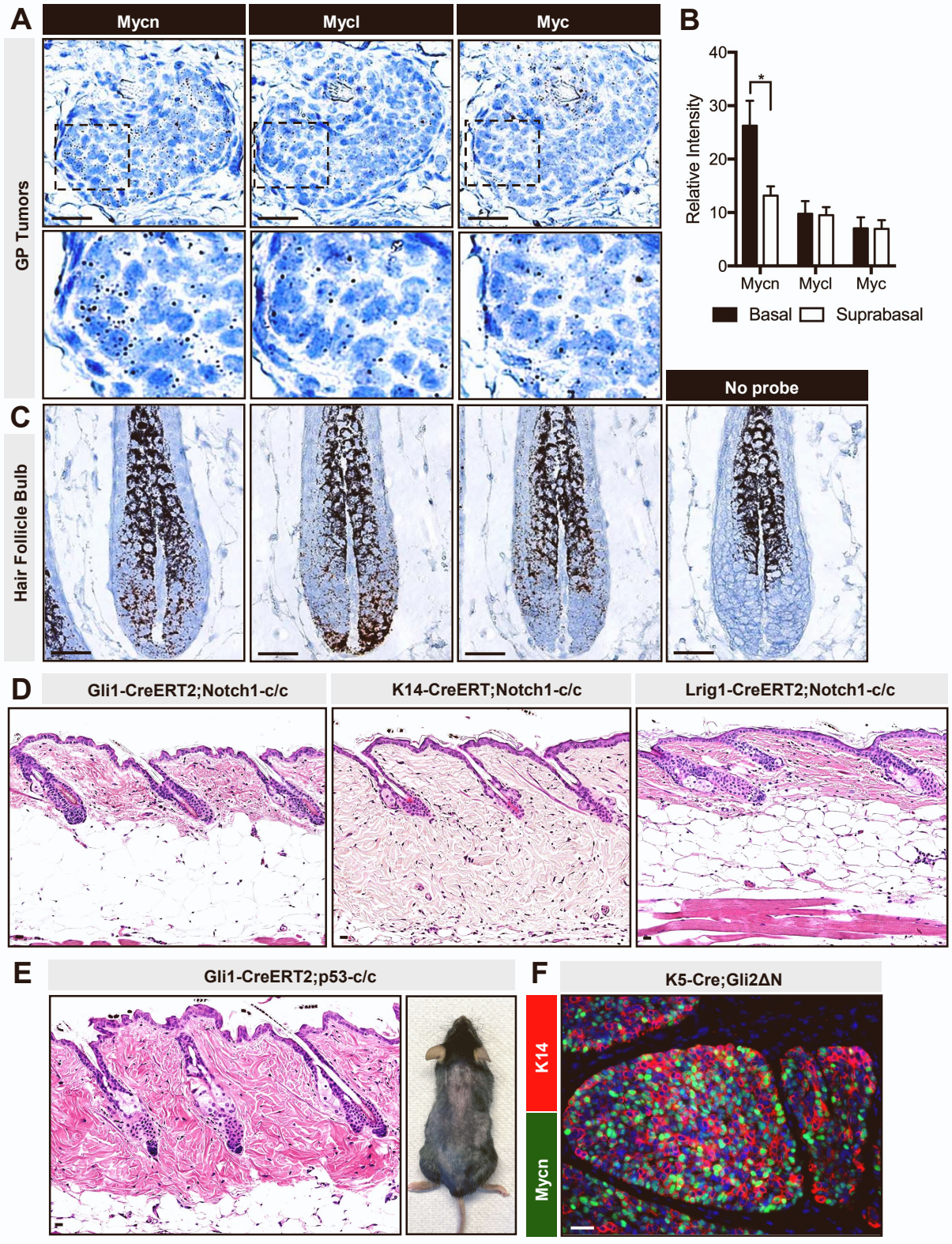
A
Mycn
Mycl
Myc
GP Tumors
B
Relative Intensity
40
30
20
10
0
*
Mycn
Mycl
Myc
Basal
Suprabasal
C
Hair Follicle Bulb
No probe
D
Gli1-CreERT2;Notch1-c/c
K14-CreERT;Notch1-c/c
Lrig1-CreERT2;Notch1-c/c
E
Gli1-CreERT2;p53-c/c
F
K5-Cre;Gli2ΔN
K14
Mycn

Figure S6. Expression of Myc family proteins in GP tumors and lack of tumor formation in *Notch1*- or *p53*-deficient skin. Related to Figure 7. A. *In situ* staining for *Mycn*, *Mycl* and *Myc* in GP tumors. Bottom panels are magnified views of the boxed areas. **B.** Quantitation of mRNA in tumor basal and suprabasal compartments. **C.** *In situ* staining for *Mycn*, *Mycl* and *Myc* in normal anagen hair follicle bulb. Right image, staining with probe omitted, showing only background staining for hair shaft and melanocytes. **D.** Histology of *Notch1*-deleted skin using 3 different inducible Cre drivers, as indicated, 15-20 weeks post-TAM. **E.** Histology of skin where *p53* was deleted from Gli1+ hair follicle stem cells, 25 weeks post-TAM. Right, photo of mouse with shaved dorsal skin devoid of palpable tumors. **F.** Mycn (green) is highly expressed in macroscopic BCC-like tumors following overexpression of a constitutively active form of Gli2 (Gli2ΔN). Data are represented as mean ± SEM, with statistics calculated by an unpaired *t*-test. *, $p < 0.05$. Scale bar, 50 μm.

Table S1. Summary of results from *Ptch1*-deficient mice

Genotype	Total # of mice	# of mice with macro tumors within 17 weeks post-TAM	Total # of mice with macro tumors (any age)	Total # of macro tumors collected*	# of Type 1 macro tumors	# of Type 2 macro tumors	# of Type 3 macro tumors	# of macro tumors with Other subtype
GP (Gli1-CreERT2 + Ptch1-c/c)	18	0	0	0	-	-	-	-
GP + Notch1-c/c (GPN1)	25	6	12	24	9	1	14	0
GP + Notch1-c/+ (GPN1-Het)	9	0	0	0	-	-	-	-
GP + Trp53-c/c (GPP53)	14	9	14	30	22	7	4	2
GP + Trp53-c/+ (GPP53-Het)	11	1	3	3	2	0	1	0
GP + rtTA + TRE-MYCN (GPT)	16	4	4	4	4	0	1	0

***** Tumors that contained ~50/50 mixed histological subtypes were scored as positive for both subtypes.

Table S2. Summary of results from other mice

Genotype	Total # of mice	Weeks post-TAM assessed for tumors	Total # of mice with tumors
Gli1-CreERT2 + Notch1-c/c	9	21-28	0
K14-CreERT + Notch1-c/c	7	20	0
Lrig1-CreERT2 + Notch1-c/c	5	15	0
Gli1-CreERT2 + Trp53-c/c	11	17-25	0
Gli1-CreERT2 + rtTA + TRE-MYCN	4	12-20	0