Figure S1

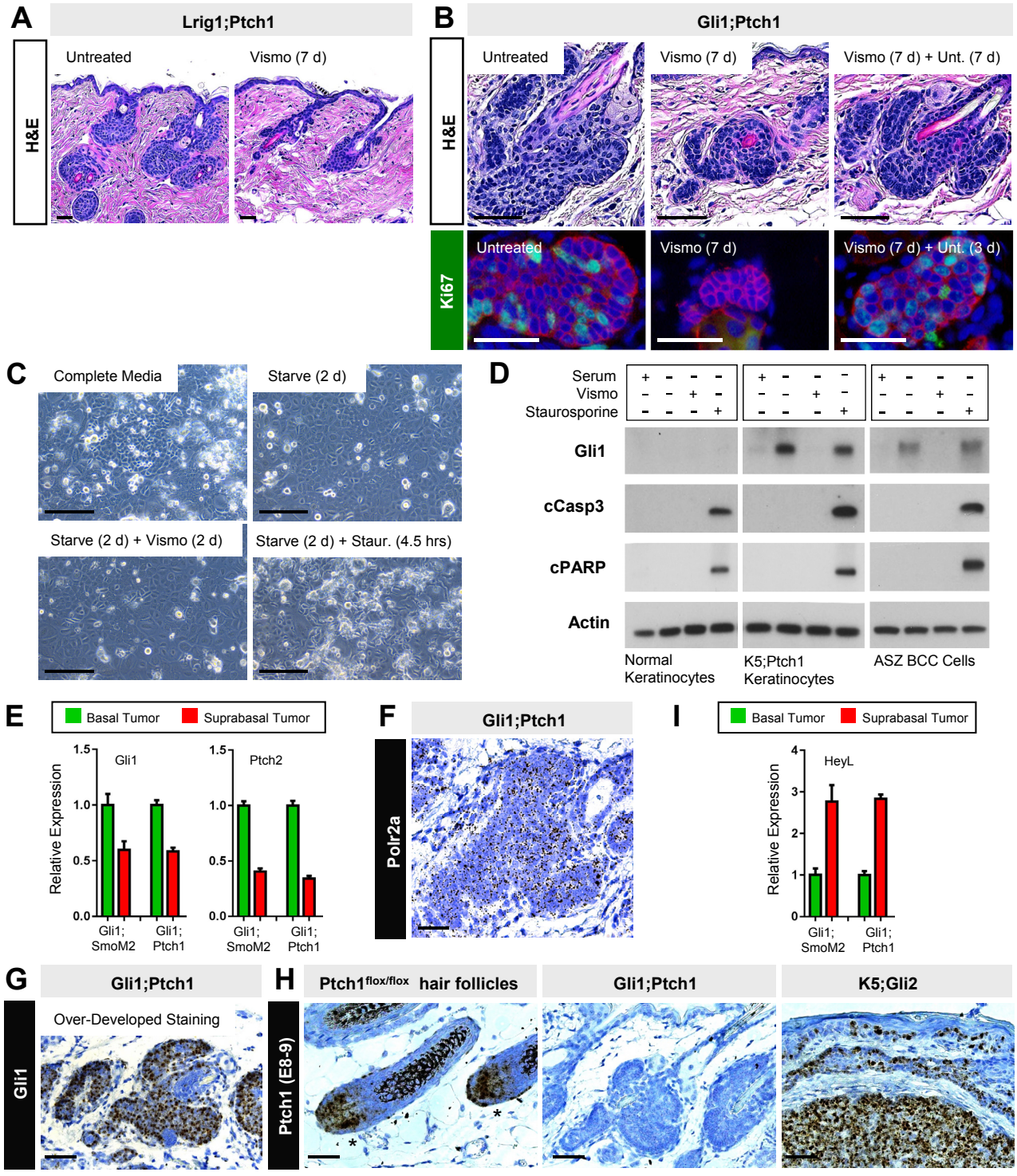

A
Lrig1;Ptch1
H&E
Untreated
Vismo (7 d)
B
Gli1;Ptch1
H&E
Untreated
Vismo (7 d)
Vismo (7 d) + Unt. (7 d)
Ki67
Untreated
Vismo (7 d)
Vismo (7 d) + Unt. (3 d)
C
Complete Media
Starve (2 d)
Starve (2 d) + Vismo (2 d)
Starve (2 d) + Staur. (4.5 hrs)
D
Serum
Vismo
Staurosporine
Gli1
cCasp3
cPARP
Actin
Normal Keratinocytes
K5;Ptch1 Keratinocytes
ASZ BCC Cells
E
Basal Tumor
Suprabasal Tumor
Relative Expression
Gli1
Ptch2
Gli1; SmoM2
Gli1; Ptch1
F
Gli1;Ptch1
Polr2a
I
Basal Tumor
Suprabasal Tumor
HeyL
Relative Expression
Gli1; SmoM2
Gli1; Ptch1
G
Gli1;Ptch1
Gli1
Over-Developed Staining
H
Ptch1flox/flox hair follicles
Gli1;Ptch1
K5;Gli2
Ptch1 (E8-9)

Figure S1. Vismo regresses tumors *in vivo*, but does not induce apoptosis in adherent cultured cells. Related to Figure 1. A. Tumors induced by *Ptch1* deletion in Lrig1^{+} hair follicle stem cells (*Lrig1-Cre^{ERT2} + Ptch1$^{flox/flox}$*, or *Lrig1;Ptch1*) (left box) can be regressed by vismo (right box), similar to *Gli1;Ptch1* tumors. **B.** Top row, histology showing *Gli1;Ptch1* tumors before (left box) and after vismo treatment (middle box). Right box, recurrent tumors, which form after vismo treatment is halted for 7 days. Bottom row, Ki67 staining (green) showing that tumor cells do not proliferate during vismo, but re-enter the cell cycle after treatment is stopped for 3 days (bottom right box). Red, co-staining for K5. Unt., untreated. **C.** Light microscopy showing primary keratinocytes expressing *Keratin 5* promoter-driven *Cre* and *Ptch1$^{flox/flox}$* alleles (*K5;Ptch1* cells) grown under different cell culture conditions, as indicated. Staur., staurosporine. **D.** Western blots for the indicated proteins, under the indicated treatment conditions. The Hh pathway is activated in starved cells and suppressed by vismo, as assessed by GLI1. Staurosporine, but not vismo, directly induces cell death, as assessed by cCasp3 and cPARP induction. Identical results were seen in *K5;Ptch1* and ASZ BCC cells. **E.** Quantitation of *Gli1* (left) and *Ptch2* (right) single molecule *in situ* staining. The average signal per cell in the tumor suprabasal compartment was normalized to the average tumor basal cell signal, which was set to '1.' **F.** *In situ* staining for the ubiquitous gene *Polr2a* confirmed diffuse expression throughout the tumor. **G.** Over-developed single molecule *in situ* staining for *Gli1* showing that both the tumor basal and suprabasal compartments possess elevated Hh target gene expression, relative to surrounding non-tumor cells. **H.** *In situ* staining for exons 8-9 of *Ptch1*, showing that these exons are present in *Ptch1$^{flox/flox}$* hair follicles that lack Cre expression (left box, asterisks), completely deleted in *Gli1;Ptch1* tumors (middle box), and elevated in tumor samples that overexpress mouse GLI2 (right box). **I.** Quantitation of *HeyL in situ* staining, similar to (E). Error bars, SE. Scale bars, 100 μm.

Figure S2

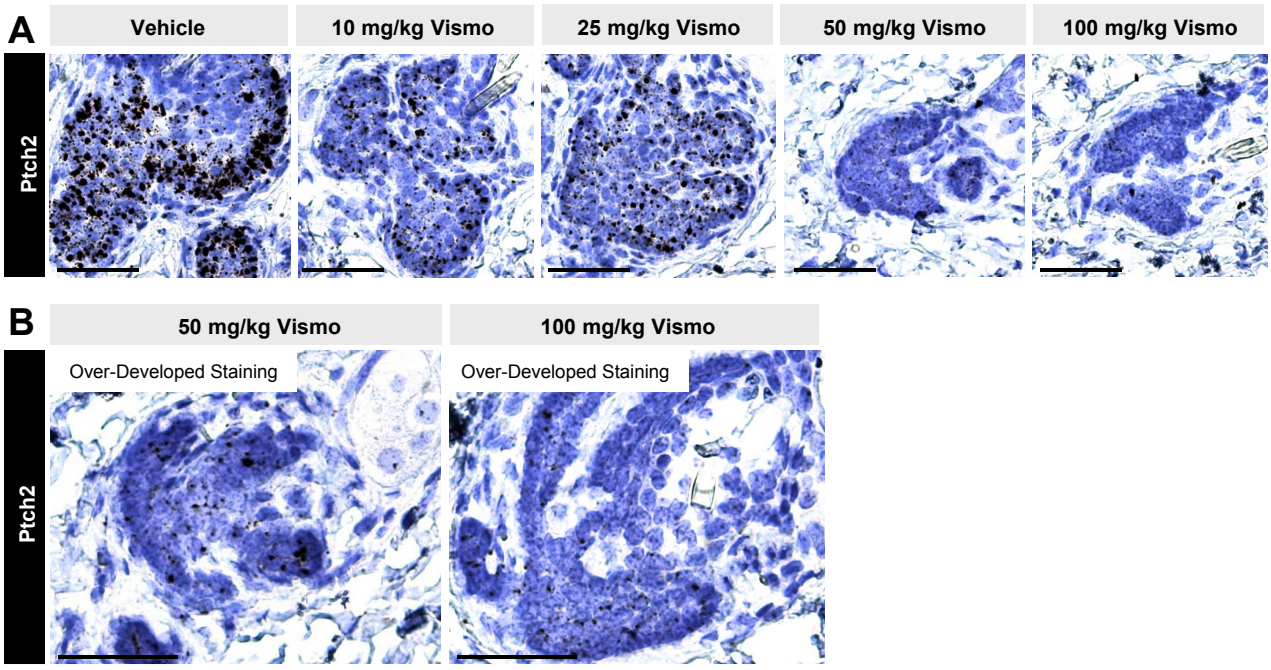


Figure S2. Vismo induces a dose-dependent reduction in Hh target gene expression. Related to Figure 3. A. *In situ* staining for *Ptch2* in *Gli1;Ptch1* mice treated with different doses of vismo for 7 days, as indicated. **B.** Over-developed *in situ* staining reveals that even at the highest doses of vismo, residual Hh target gene expression can still be detected. Scale bars, 100 μm.

Figure S3

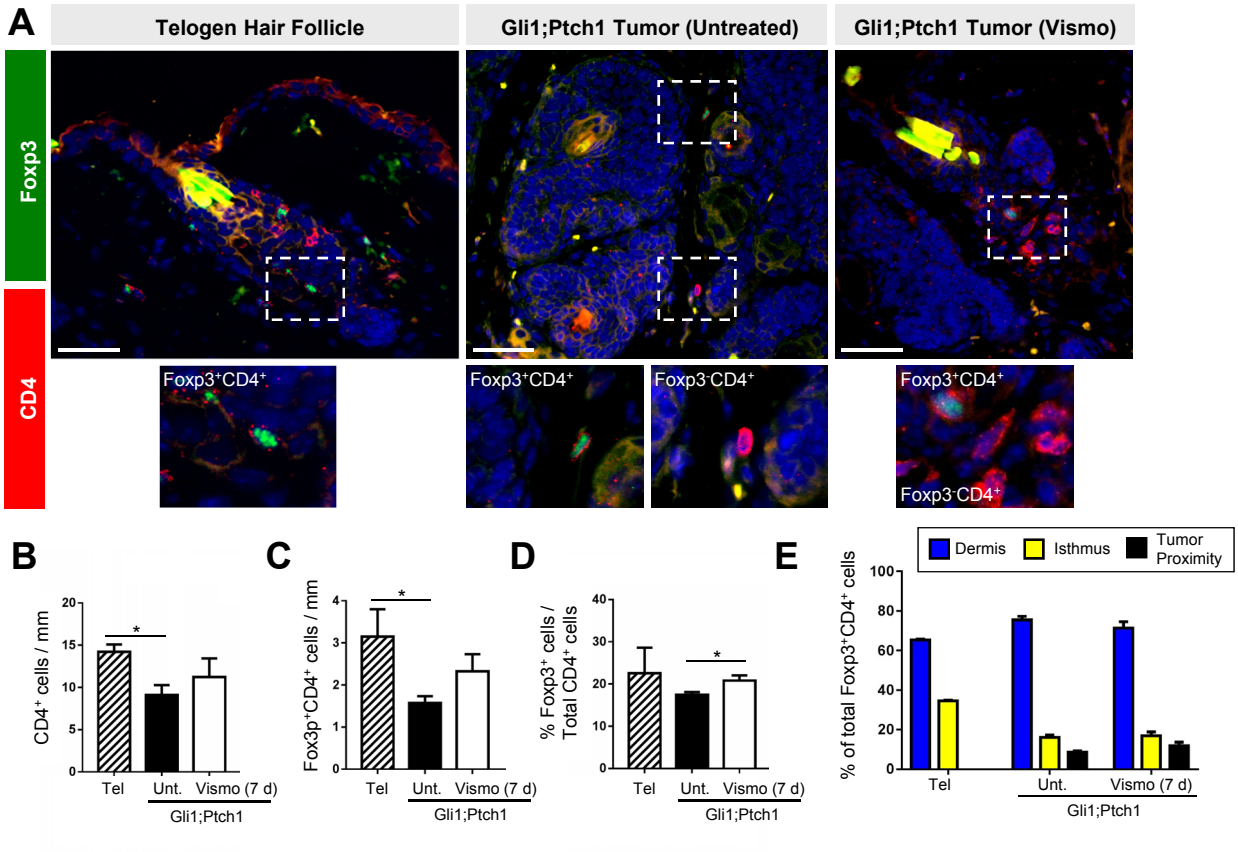


Figure S3. Abundance and localization of regulatory T cells (Tregs) are largely unchanged in tumors before and after vismo treatment. Related to Figure 3. A. Immunohistochemistry showing that nuclear Foxp3$^+$ Tregs (green) represent a subset of CD4$^+$ T cells (red). Shown are stainings from normal telogen mouse skin (left), untreated *Gli1;Ptch1* skin (middle), and 7 day vismo-treated *Gli1;Ptch1* skin (right). Bottom images are magnified views of the boxed areas. Tregs were enriched in the hair follicle isthmus and dermis and, to a lesser extent, near the bulge. Tregs were observed in the tumor proximity (defined here as within 20 µm of the tumor edge), but were never observed inside tumors. **B.** Quantitation of total CD4$^+$ T cell abundance, per mm of skin section. Tel, normal telogen skin. Unt., untreated tumors. **C.** Quantitation of total Foxp3$^+$ Treg abundance, per mm of skin section. **D.** Proportion of total CD4$^+$ T cells that were Foxp3$^+$ Tregs. **E.** Localization of Tregs in dermis, hair follicle isthmus or in proximity (within 20 µm) of the tumor edge. Tregs appeared reduced in tumor samples compared to normal skin, possibly because these immune cells were never observed within tumor masses, which occupied a substantial fraction of the total skin section area. Error bars, SE. *, $p < 0.05$. Scale bars, 100 µm.

Figure S4

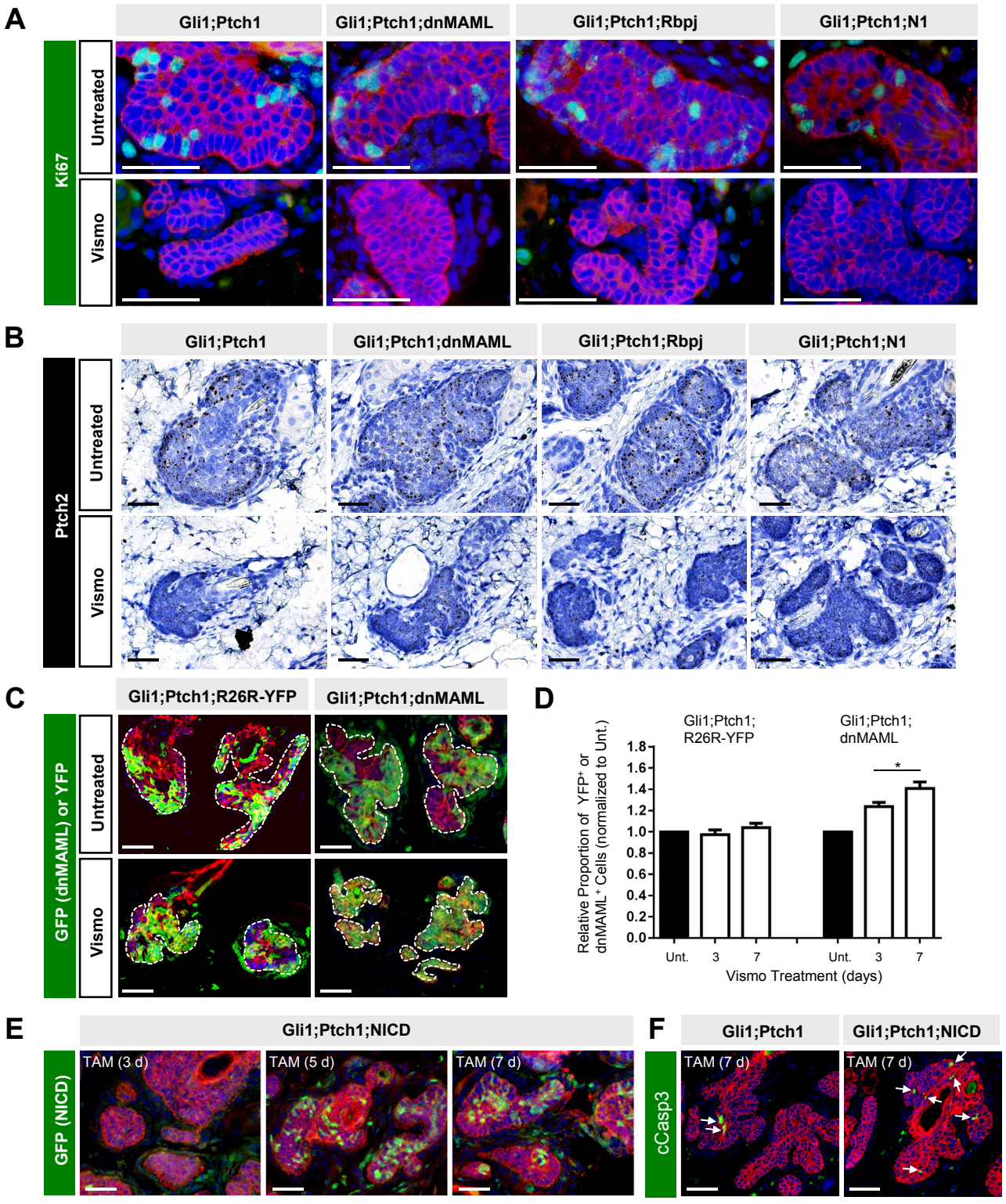

A
Gli1;Ptch1
Gli1;Ptch1;dnMAML
Gli1;Ptch1;Rbpj
Gli1;Ptch1;N1
Ki67
Untreated
Vismo
B
Gli1;Ptch1
Gli1;Ptch1;dnMAML
Gli1;Ptch1;Rbpj
Gli1;Ptch1;N1
Ptch2
Untreated
Vismo
C
Gli1;Ptch1;R26R-YFP
Gli1;Ptch1;dnMAML
GFP (dnMAML) or YFP
Untreated
Vismo
D
Gli1;Ptch1; R26R-YFP
Gli1;Ptch1; dnMAML
Relative Proportion of YFP+ or dnMAML+ Cells (normalized to Unt.)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Unt.
3
7
Unt.
3
7
Vismo Treatment (days)
E
Gli1;Ptch1;NICD
GFP (NICD)
TAM (3 d)
TAM (5 d)
TAM (7 d)
F
Gli1;Ptch1
Gli1;Ptch1;NICD
cCasp3
TAM (7 d)
TAM (7 d)

Figure S4. Notch inhibition does not affect cell proliferation or Hh activity, while Notch activation induces tumor cell death. Related to Figure 5. A. Cell proliferation (green, top row) is suppressed in all *Gli1;Ptch1* tumors regardless of Notch status, upon vismo treatment (bottom row). **B.** Hh pathway activity, as assessed by *in situ* staining for *Ptch2*, is unchanged in tumors regardless of Notch status (brown, top row), and largely suppressed upon 7 days of vismo (bottom row). **C.** *Gli1;Ptch1* tumor cells expressing dnMAML (*Gli1;Ptch1;dnMAML*, right boxes, green) increase in relative abundance upon vismo treatment for 7 days. In contrast, *Gli1;Ptch1* tumor cells expressing a *ROSA26* promoter-driven *YFP* reporter allele (*Gli1;Ptch1;R26R-YFP*, left boxes, green) do not increase in relative abundance upon vismo. In all cases, not all tumor cells express dnMAML or the YFP reporter due to incomplete Cre-mediated recombination. **D.** Quantitation for C. Unt., untreated. **E.** *Gli1;Ptch1* tumors harboring an activated Notch1 allele (*Gli1;Ptch1;NICD*) do not express the transgene in tumors, as monitored by IRES-GFP expression, 5 weeks after tamoxifen (see also Figure 5F). Upon boosting with tamoxifen chow (TAM) for 5-7 days, expression of GFP, indicating expression of activated Notch1 (green), is detected. **F.** Tamoxifen-boosted *Gli1;Ptch1;NICD* tumors display more apoptotic cells (green, arrows) than do tamoxifen-boosted *Gli1;Ptch1* control tumors. Red, co-staining for K17 (Figure S4C) or K5 (all other panels). Error bars, SE. *, $p < 0.05$. **, $p < 0.01$. Scale bars, 100 μm.

Figure S5

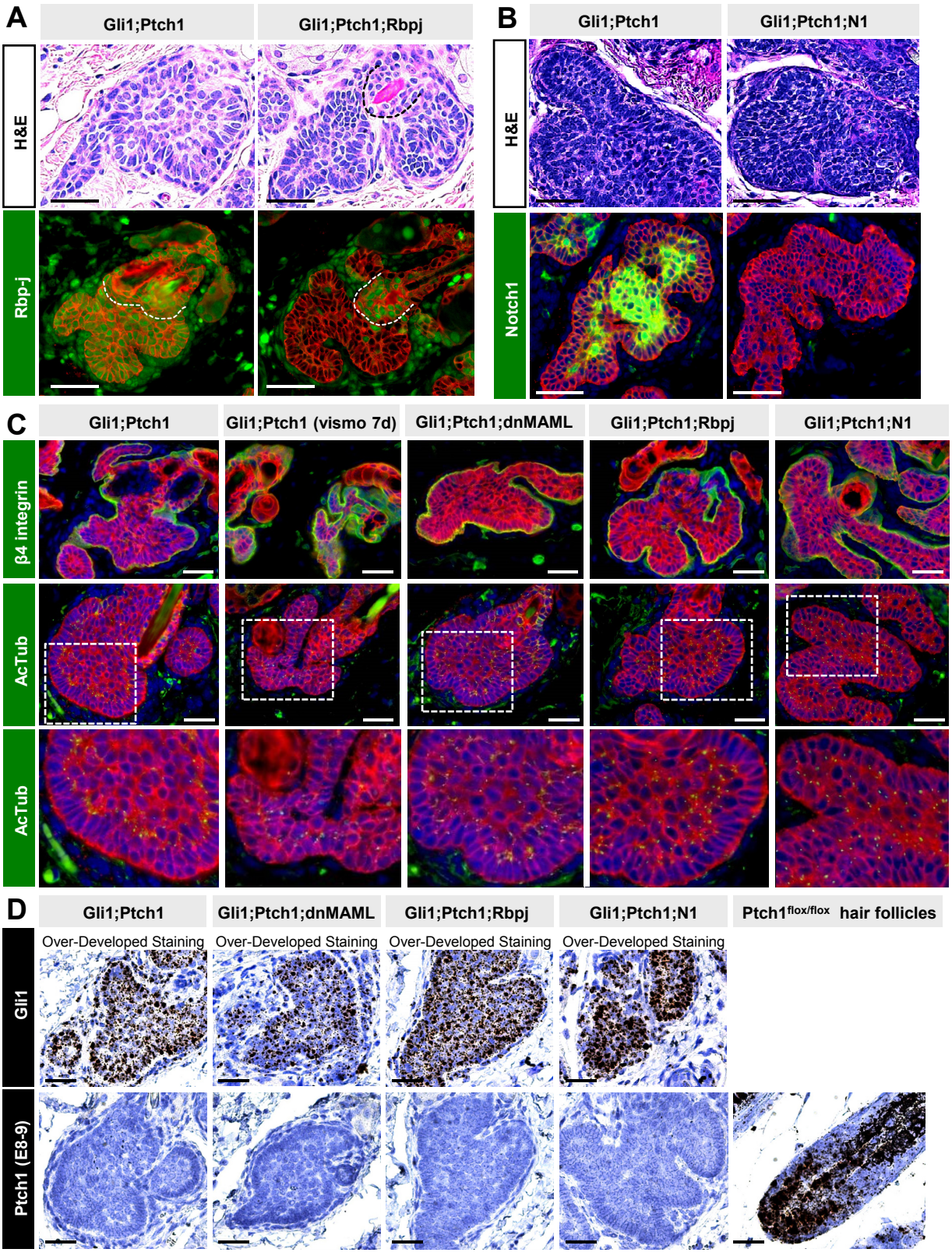
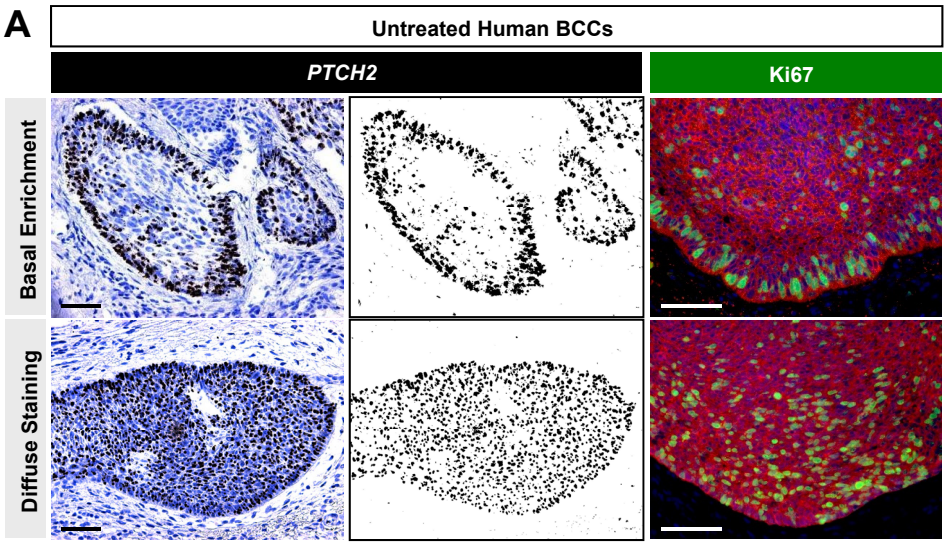
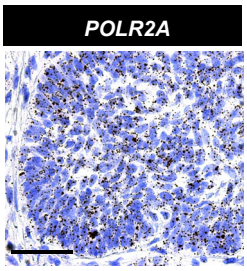
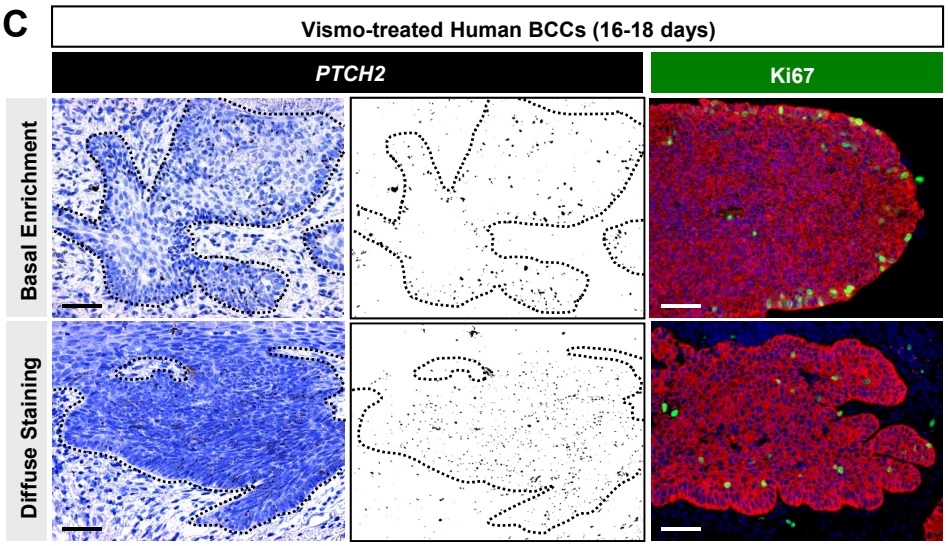

A
Gli1;Ptch1
Gli1;Ptch1;Rbpj
H&E
Rbp-j
B
Gli1;Ptch1
Gli1;Ptch1;N1
H&E
Notch1
C
Gli1;Ptch1
Gli1;Ptch1 (vismo 7d)
Gli1;Ptch1;dnMAML
Gli1;Ptch1;Rbpj
Gli1;Ptch1;N1
β4 integrin
AcTub
AcTub
D
Gli1;Ptch1
Gli1;Ptch1;dnMAML
Gli1;Ptch1;Rbpj
Gli1;Ptch1;N1
Ptch1flox/flox hair follicles
Over-Developed Staining
Over-Developed Staining
Over-Developed Staining
Over-Developed Staining
Gli1
Ptch1 (E8-9)

Figure S5. Rbpj and Notch1 are efficiently deleted in *Gli1;Ptch1* tumors, without causing abnormal cell polarity. Related to Figure 6. A. Top, histology of *Gli1;Ptch1* (left) and *Gli1;Ptch1;Rbpj* tumors (right). Bottom, IHC showing efficient loss of RBP-J (green) in *Gli1;Ptch1;Rbpj* tumors. Dotted lines, hair follicle bulge. **B.** Top, histology of *Gli1;Ptch1* (left) and *Gli1;Ptch1;N1* tumors (right). Bottom, IHC showing efficient loss of Notch1 (green) in *Gli1;Ptch1;N1* tumors. **C.** Staining for β4 integrin (top row) and acetylated tubulin (AcTub, middle and bottom rows) showed that disrupting Notch does not affect tumor cell polarity. Note the apical localization of cilia (green) and basal localization of β4 integrin (green) in basal tumor cells irrespective of Notch status. Bottom row images are magnified views of the boxed areas. Red, K5 co-staining. **D.** Top, over-developed *in situ* staining for *Gli1* shows that the Hh pathway is activated throughout the tumor irregardless of Notch status. Bottom, probes specific for exons 8-9 of *Ptch1* detected no signal in these tumors. This confirms that all cells within the tumor had deleted *Ptch1*; that both tumor basal and suprabasal cells upregulate Hh signaling, relative to surrounding normal tissue; and that basal tumor cells, in particular, display the highest levels of Hh pathway activation (similar to Figure 3F-G). Scale bars, 100 μm.

Figure S6







D

Tumor #	Vismo (days)	Hh Levels	Basal Hh	Ki67	Basal Ki67
MF3	0	+++	50%	+++	50%
MF4	0	+++	80%	+++	80%
MF9	0	+++	N/A*	+++	N/A*
MF10	0	+++	80%	+++	80%
MF18	0	+++	0%	+++	60%
MF12	18	Undetectable	N/A	Undetectable	N/A
MF14	18	Undetectable	N/A	Undetectable	N/A
MF19	18	+	0%	+	20%
MF20	16	+	80%	+	80%
MF21	16	+	80%	+	50%

Figure S6. Some human BCCs display basally enriched Hh pathway activation and proliferation, both in the absence or presence of vismo. Related to Figure 7. A. Untreated BCCs from a second cohort, independent from samples shown in Figure 7, displayed basal enrichment of the Hh target gene *PTCH2* and cell proliferation (green) (top row), or in other cases displayed diffuse staining throughout the tumor (bottom row). Black and white images are also shown for *PTCH2* staining, where the nuclear counterstain was digitally removed to improve clarity. **B.** *POLR2A* exhibits diffuse tumor staining, even in untreated tumors where Hh activation was found to be enriched at the periphery. **C.** Vismo-responsive BCCs treated for 16-18 days showed residual staining for *PTCH2*. In some tumors, *PTCH2* was again enriched at the periphery (top row), or diffusely expressed (bottom row). Lingering proliferative cells (green), either enriched at the periphery or diffusely distributed throughout the tumor, were also observed. Red, co-staining for K5. **D.** Table summarizing staining results. Percentages are a visual estimate of the total tumor periphery within a sample that displayed basally enriched Hh or Ki67. For example, "80%" indicates basally-enriched staining in 80% of the tumor, and diffuse staining in the remaining 20%, for the indicated marker. N/A, not applicable. *, note that BCC sample MF9 was entirely comprised of thin tumor strands, with few interior suprabasal cells. Scale bars, 100 μm.

TABLE S1

Quantitative PCR Primers (Related to Key Resources Table)

Target	Forward Primers (5' – 3')	Reverse Primers (5' – 3')
α6-integrin	AGACCAGTGGATGGGAGTCA	ACGTGCTGCCGTTTCTCATA
EYFP (SmoM2)	CTGACCCTGAAGTTCATCTGC	GTGCGCTCCTGGACGTAG
Gli1	TTGGGATGAAGAAGCAGTTG	GGAGACAGCATGGCTCACTA
Hes1	ACACCGGACAAACCAAAGAC	CTTCGCCTCTTCTCCATGAT
Hey1	GCAGATGACTGTGGATCACC	GGGATGCGTAGTTGTTGAGA
Hey2	AAGATGCTCCAGGCTACAGG	TGTCAAGCACTCTCGGAATC
HeyL	CGTGGATCACTTGAAGATGC	CATTCCCGAAACCCAATACT
Hhip	TCGGGTCACATCTTGGGATTTGG	GGAGCAATCGGAGGTCAGTGG
Hprt	AGGACCTCTCGAAGTGTTGGATAC	AACTTGCGCTCATCTTAGGCTTTG
Jag1	AGGCGTCCTCTGAAAAACAG	TTGTTGGTGGTGTTGTCCTC
Jag2	CCTCCTGCTGCTTTGTGAT	GAGTGATAGCAGCCACGATG
Krt17	GAGAAGATGGCGGAGAAGAA	CCCTCCAGAGATGCTTTCAT
Krt14	CTCTGGCTCTCAGTCATCCA	GACAATACAGGGGCTCTTCC
Ptch1	CTGCTGTGGTGGTGGTATTC	GGCTTGTGAAACAGCAGAAA
Ptch2	GGTCCTCCGCACCTCATATC	CTGTCTCAATTACAGCCACTC