

Fig. S1. Non-specific recognition of Ag-7195 by an antibody generated against Gli2. **A.** H&E staining of E17.5 skin from *Gli2*^{+/-} and *Gli2*^{-/-} embryos, showing reduced hair follicle abundance and a general delay in hair follicle morphogenesis upon complete loss of *Gli2*. **B.** IHC showing that the Ag-7195 antibody recognizes an antigen (red) in E17.5 skin sections that is maintained even in *Gli2* null embryos. The basal layer marker K5 is shown in green. Scale bars, 50 μ m.

Fig. S2. Keratin gene expression profile in different sweat gland compartments. Data were extracted from a study by Lu et al., using the NCBI Gene Expression Omnibus (GEO) (accession number GSE37274)(Lu et al., 2012). Probe set ID's were downloaded from NetAffx Analysis Center. For some genes, multiple probe sets are present on the gene chip and are shown on the left column (e.g., K6A). Normalized values are displayed as a heat map generated by CIMminer, ranging from light blue (low expression) to red (high expression). Values were not log-transformed, nor was a z-score applied, in order to easily visualize genes with low signal intensity, suggesting poor expression. Most keratins in this dataset displayed relatively low signal intensity (light blue), although several well-characterized keratins (e.g. K1, 5, 6, 10, 14) displayed higher signals. The expression values for K79 are indicated by the arrow. MyoEp, myoepithelium; S-Basal, suprabasal; Palmopl Epiderm, palmoplantar epidermis.

Fig. S3. Specificity of the Ag-7195 and K79 antibodies. **A.** Western blots showing lysates from 293FT cells overexpressing vector alone ("mock"), FLAG-tagged full-length mouse *Gli2*, or K79, probed with various antibodies, as shown after short or long film exposures. Anti-FLAG antibody detected a specific band at ~185 kDa only in cells transfected with *Gli2*, as expected (top). The Ag-7195 antibody detected K79, as well as a band similar in size to *Gli2* upon long exposure (*) (middle). Importantly, an independent antibody generated specifically against K79 detected overexpressed K79, but not *Gli2*, even after long exposure (bottom). **B.** Quantitative PCR showing that K79, but not *Gli2*, is enriched in FACS-sorted suprabasal hair follicle cells from *Shh;YFP* mice. Two independent primer sets against *Gli2* were utilized, generated against either the 5' or 3' regions of the transcript.

Fig. S4. Ag-7195 is likely K79. IHC using the Ag-7195 antibody (green) and a second antibody independently generated specifically against K79 (red) reveals co-localization in the telogen sINF (also depicted in Figure 2G); in the SHG of an early anagen follicle (arrows); in suprabasal streams departing from an early hair bud; and along the suprabasal layer of a sweat gland duct. Epidermal staining in embryonic skin (red) is due to non-specific reactivity of the anti-goat secondary antibody. Scale bars, 50 μ m.

Fig. S5. K79 expression in different human tissues. Normalized signal intensity values are shown using data extracted from a study by Dezsó et al., using NCBI GEO (accession number GSE7905)(Dezsó et al., 2008). Three replicates per tissue were analyzed (blue bars, replicate 1; yellow bars, replicate 2; red bars, replicate 3). UHR, universal human reference. PBLs, peripheral blood leukocytes.

Fig. S6. Long-term lineage tracing of *Lrig1*-derived cells. Left panel, anagen follicles from an *Lrig1;YFP* mouse, 56 days after induction by tamoxifen, revealing YFP labeling (green) in the INF, but not in the IFE, bulge or lower regenerated bulb. The basal layer marker K5 is depicted in red. Middle panel, YFP single-channel view of the same anagen follicles. SG, sebaceous glands. Right panel, β -gal staining of telogen skin from an *Lrig1;LacZ* mouse, >100 days after induction by tamoxifen, revealing that the INF is stably labeled, but the IFE and bulge are both largely devoid of labeling. Scale bars, 50 μ m.

Fig. S7. K79 is not expressed in E15.5 hair follicle placodes. Serial sections, as indicated, from E15.5 *Shh;YFP* dorsal epidermis. Hair placodes express or are derived from cells that expressed *Shh*, and are labeled by YFP (green), but do not express K79 (red). Scale bars, 50 μ m.

Fig. S8. K79⁺ cells originate from E16.5 hair germs. **A.** Serial sections through an E16.5 *Shh;YFP* early hair germ, stained for YFP (green) and K79 (red). **B.** Enlarged merged and single channel views of the same hair germ. Serial section slices are as indicated. Arrows, early K79⁺YFP⁺ cells located within the hair germ, prior to outward migration. Scale bars, 50 μ m.

Fig. S9. Early migratory K79⁺ cells originate from hair germs. **A.** Serial sections, as indicated, through a more advanced E16.5 *Shh;YFP* hair germ (arrow), stained for YFP (green) and K79 (red). **B.** Enlarged merged and single channel views of the same hair germ. Serial section slices are as indicated. Arrows, early migratory K79⁺YFP⁺ cells extend in a posterior direction out of the hair germ. Section 3 is also depicted in Fig. 4B. Scale bars, 50 μ m.

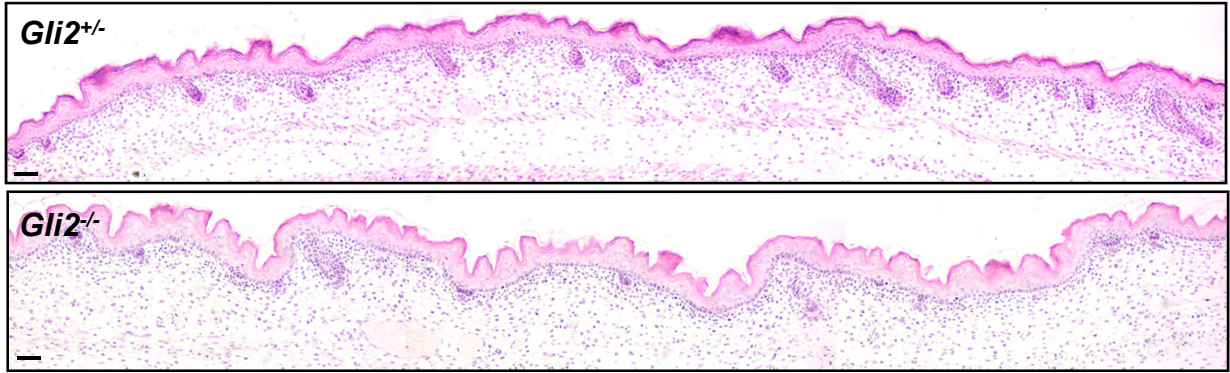
Fig. S10. Characterization of migratory hair bud-derived cells. Upper panels, IHC staining reveals that the domain of Plet-1 expression (red) initially occupies the upper hair germ and surrounding epidermis. This pattern becomes more restricted in the early hair peg, as Plet-1 co-localizes with K79 (green) within the developing follicle, although weak expression can still be detected in the surrounding epidermis. Lower panels, IHC for K17 and K10 (red, arrows) in developing hair germs, showing that suprabasal migratory streams are likely positive for both these keratins. Scale bars in upper panels, 50 μ m; in lower panels, 25 μ m.

Fig. S11. During early anagen, K79 is expressed by suprabasal SHG cells prior to and during migration along the anterior bulge. **A.** *Gli1;LacZ* follicles display labeling in the upper bulge and SHG (blue), with a gap of unlabeled bulge cells in between (dotted line), as previously reported (Brownell et al., 2011). Both high- and low-level labeling of follicles is shown (left and right panels, respectively). Arrowhead, non-specific labeling due to endogenous β -galactosidase activity. The left panel is also depicted in Fig. 5E. **B.** Merged and single channel views of early anagen follicles from *Gli1;YFP* mice. (i) $K79^+$ cells originate from the SHG during anagen I and are YFP^+ prior to migration. (ii) $K79^+YFP^+$ cells turning along the base of the anterior face of the bulge. (iii-iv) $K79^+YFP^+$ cells streaming along the anterior face of the bulge. Note the progressive narrowing of the gap of formerly unlabeled bulge cells along the anterior face (yellow dotted lines) in *Gli1;YFP* follicles by migratory $K79^+YFP^+$ cells during early anagen. Also note that $K79^+$ streams contain both YFP^+ and YFP^- cells likely due to low-level labeling of the SHG, indicating that these streams are polyclonal. Scale bars, 50 μ m.

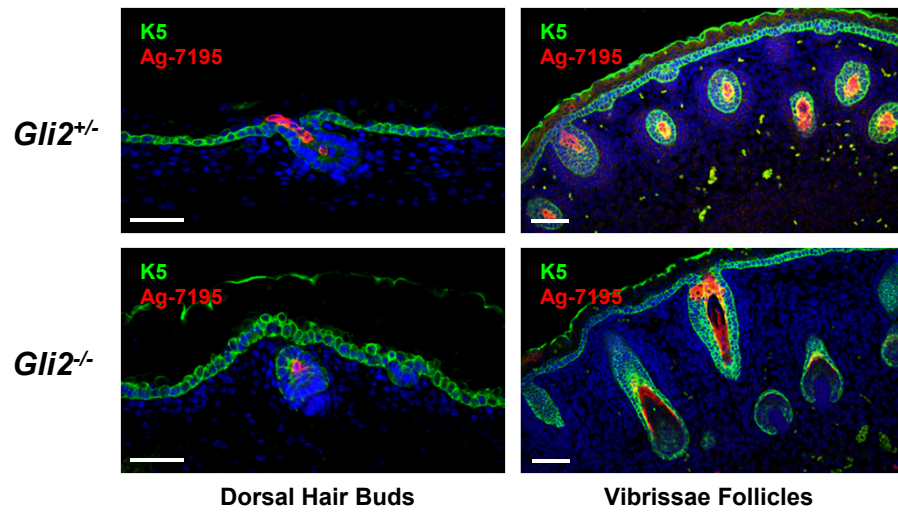
Fig. S12. Expression of dnMAML-GFP in the INF, 10 weeks after TAM induction. Upper left panel, expression of dnMAML-GFP (green) does not cause overt INF disruption or affect K79 expression (red), 10 weeks after tamoxifen-mediated induction of adult *Lrig1;dnMAML* mice. Note the abnormal egress of dnMAML-GFP-expressing cells from the INF into the IFE (arrow). Upper right panel, age-matched littermate control skin sample. Lower panels, magnified single-channel views of the region indicated by (*). Scale bars, 50 μ m.

Veniaminova_Fig S1

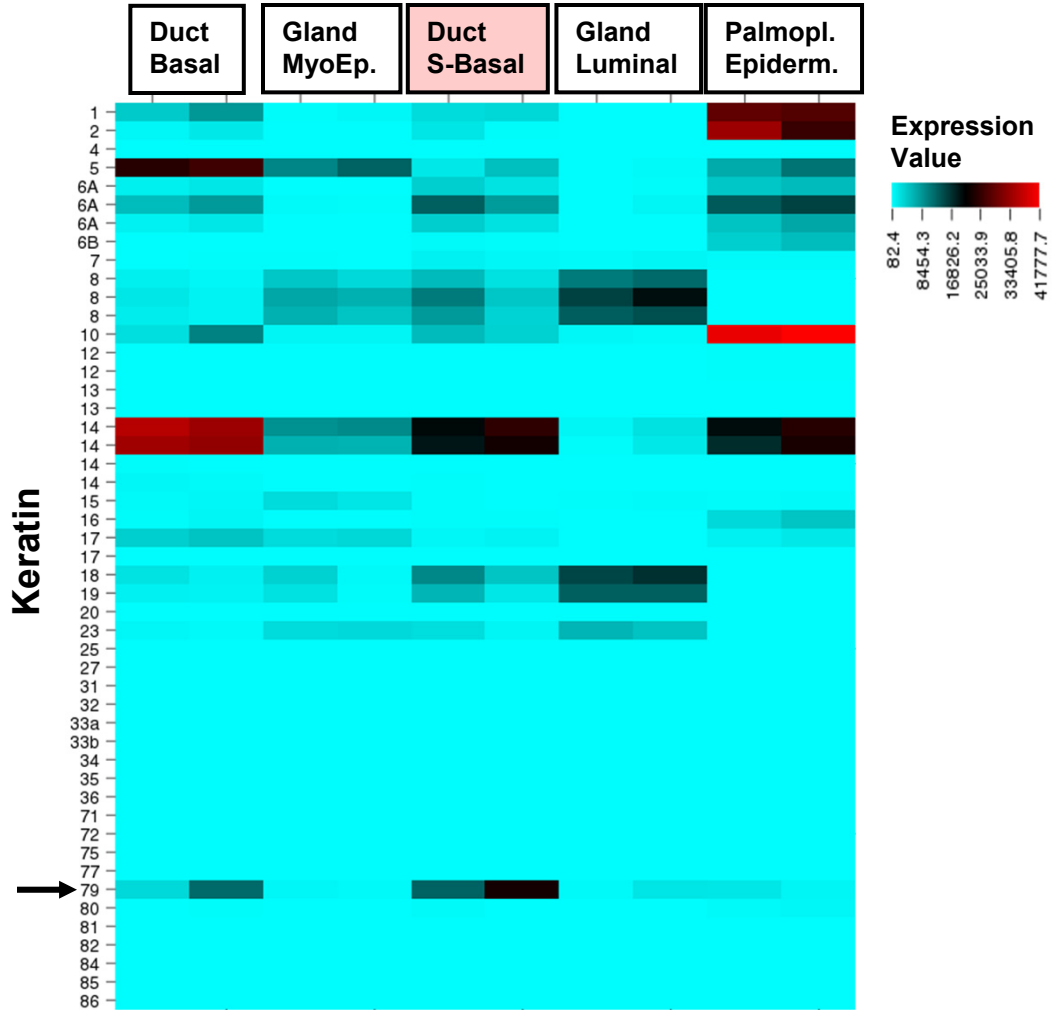
A



B

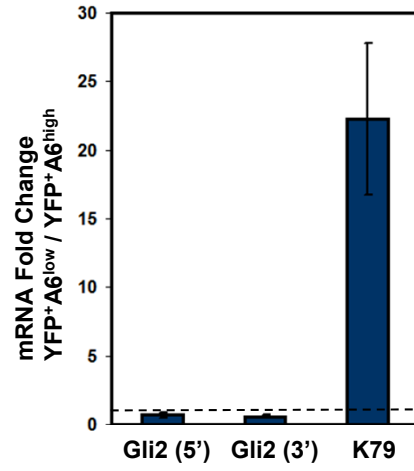


Veniaminova_Fig S2

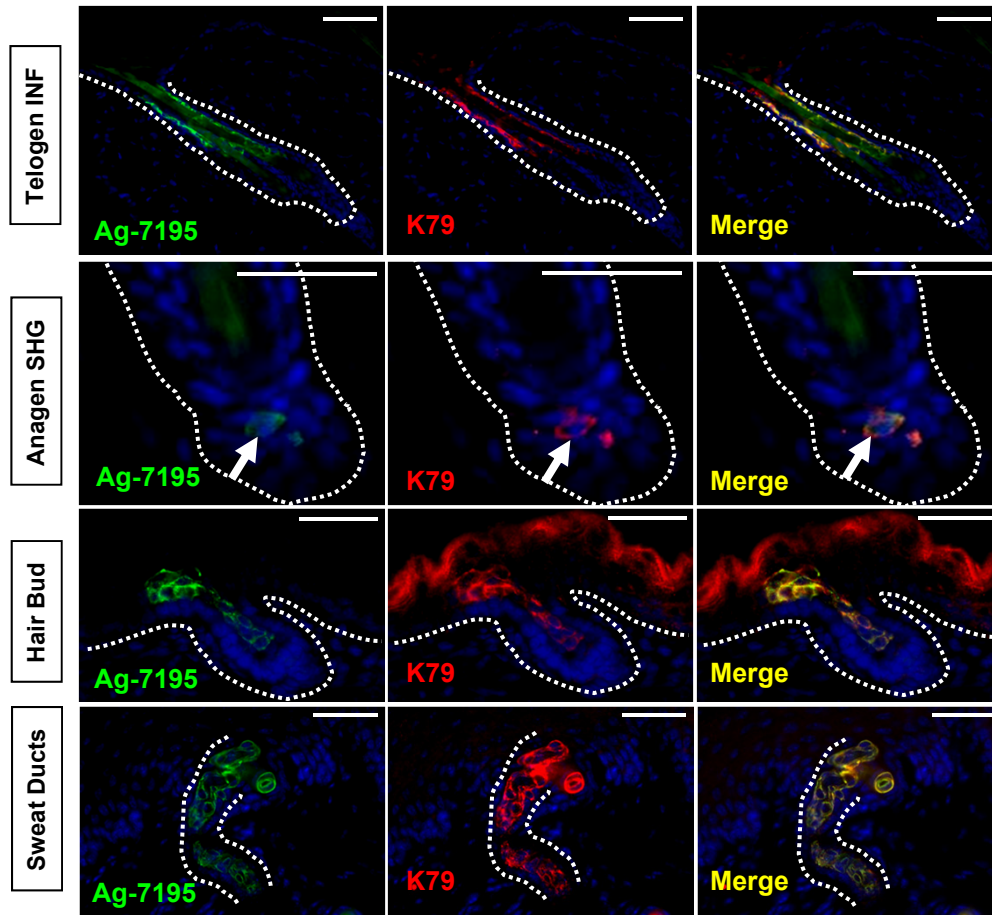


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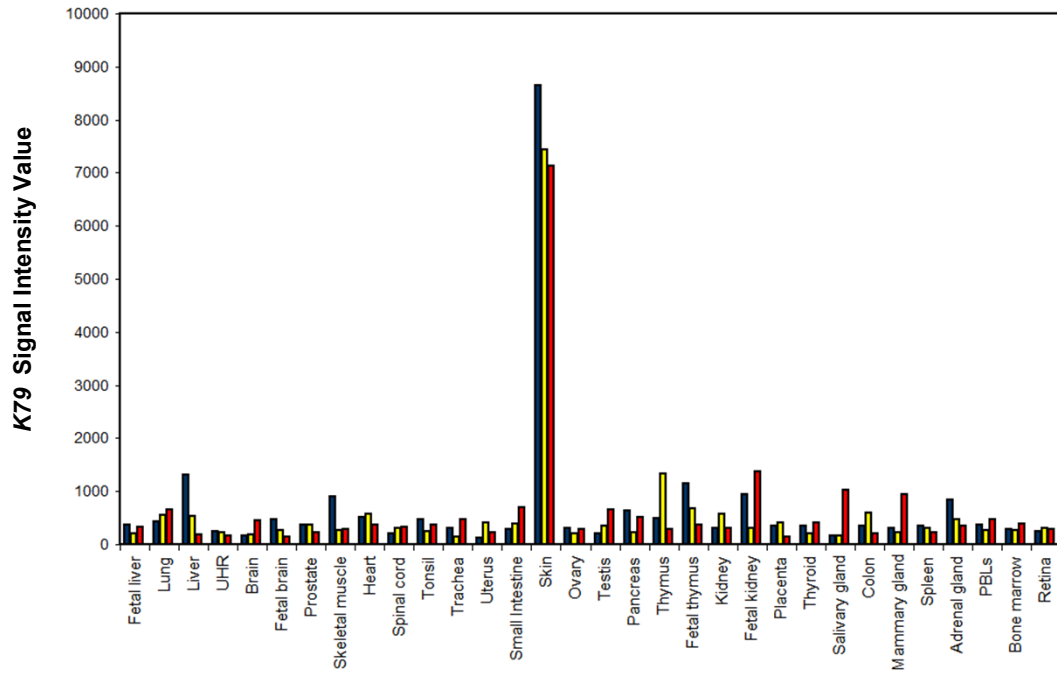
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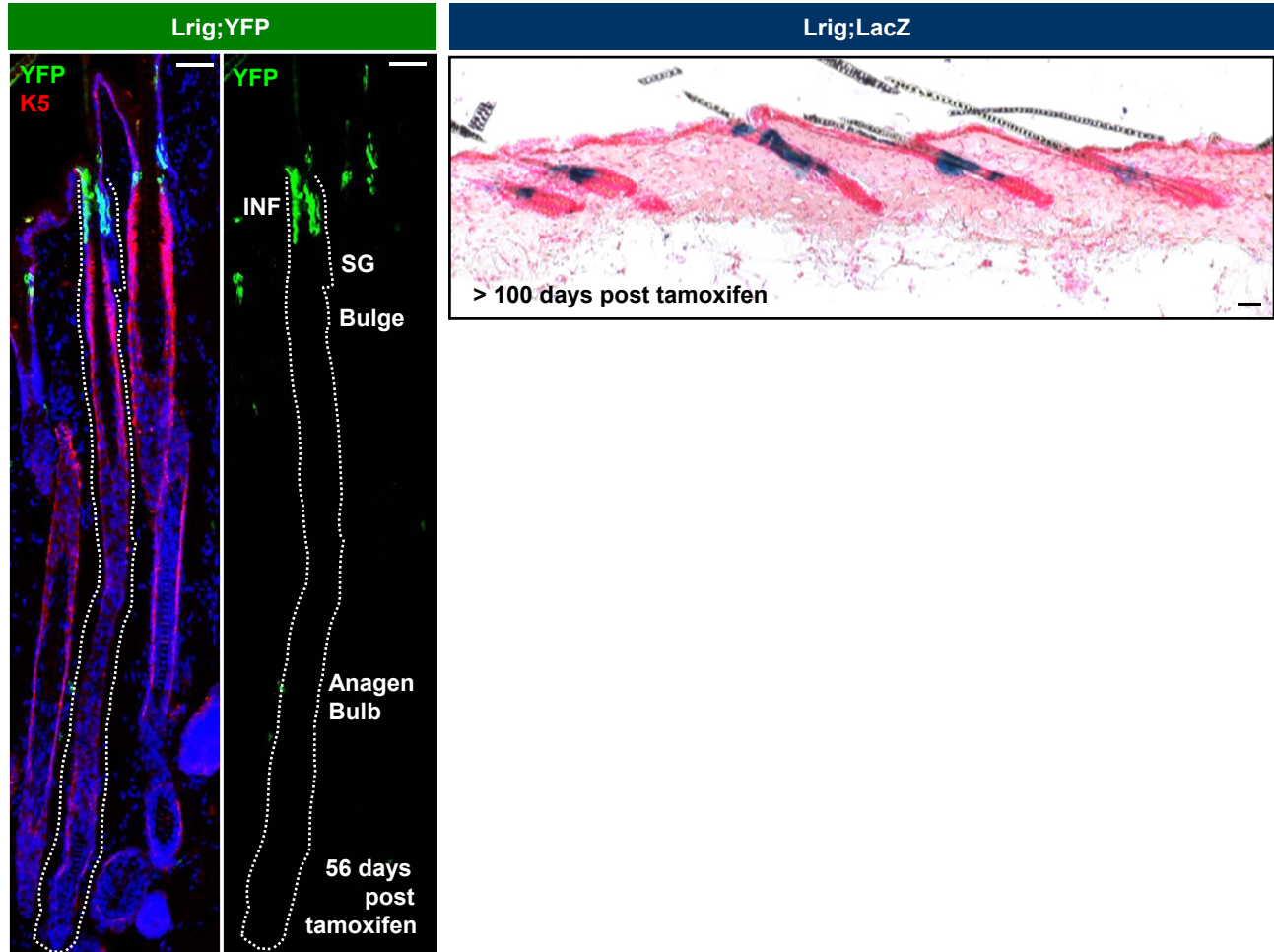
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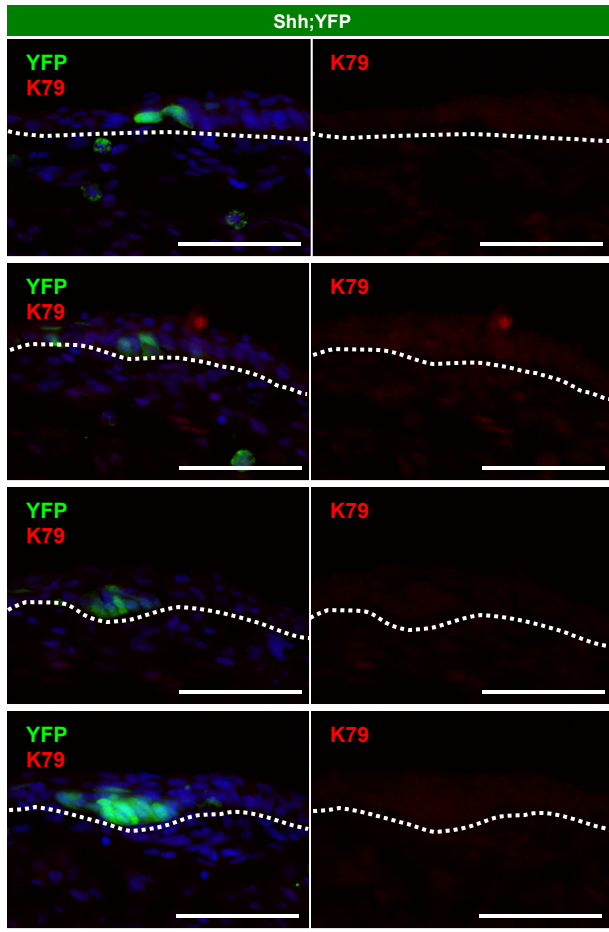
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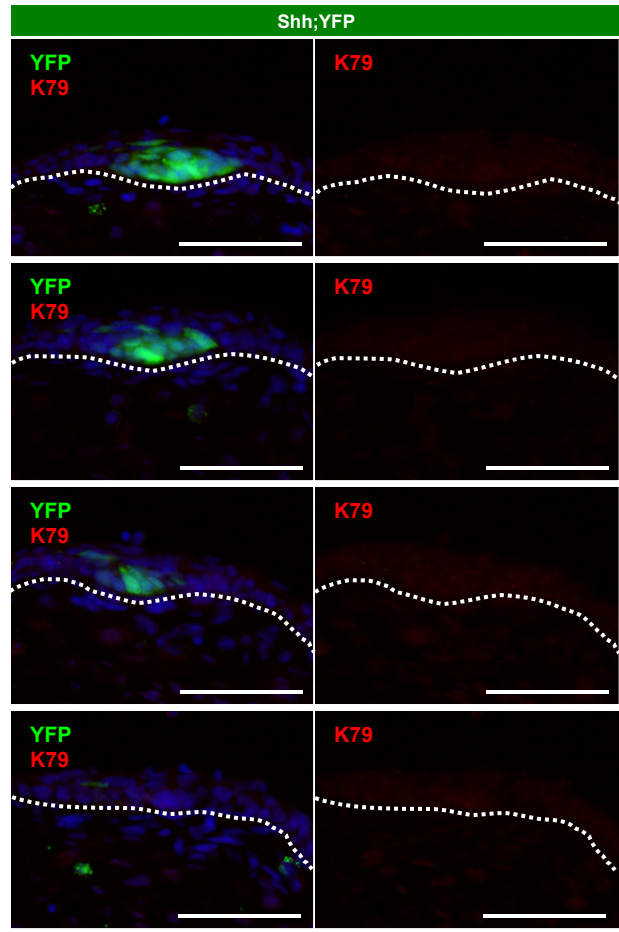
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Veniaminova_Fig S7



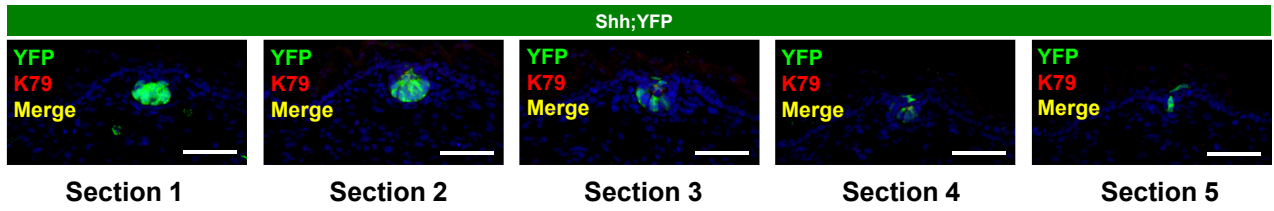
Serial sections #1-4 (top to bottom)
E15.5 embryonic dorsal epidermis



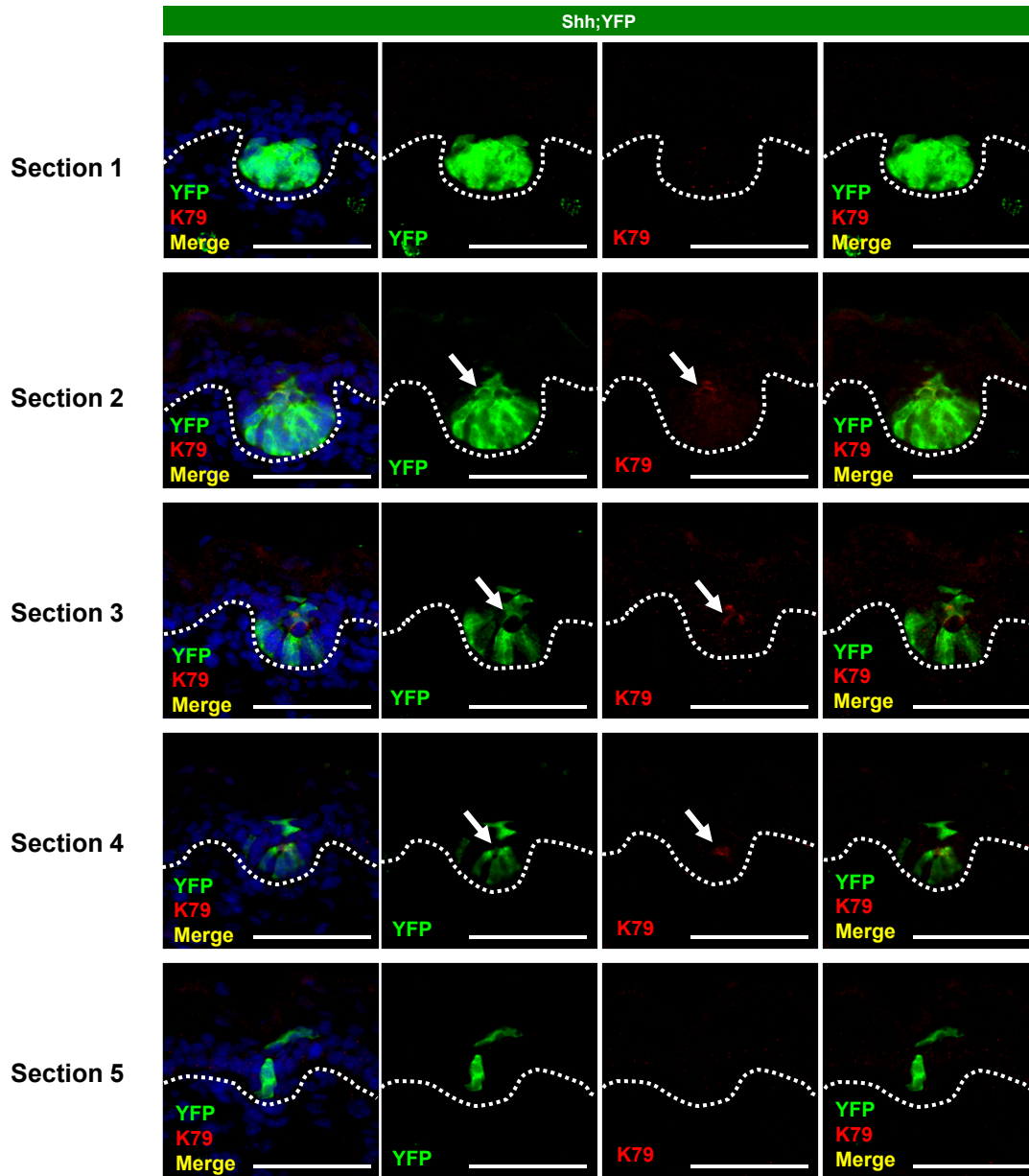
Serial sections #5-8 (top to bottom)
E15.5 embryonic dorsal epidermis

Veniaminova_Fig S8

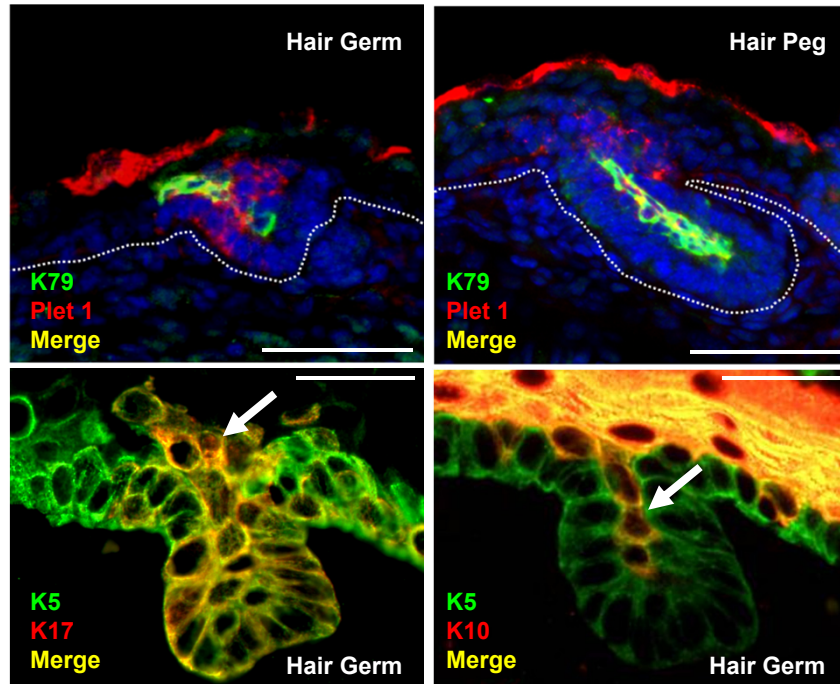
A



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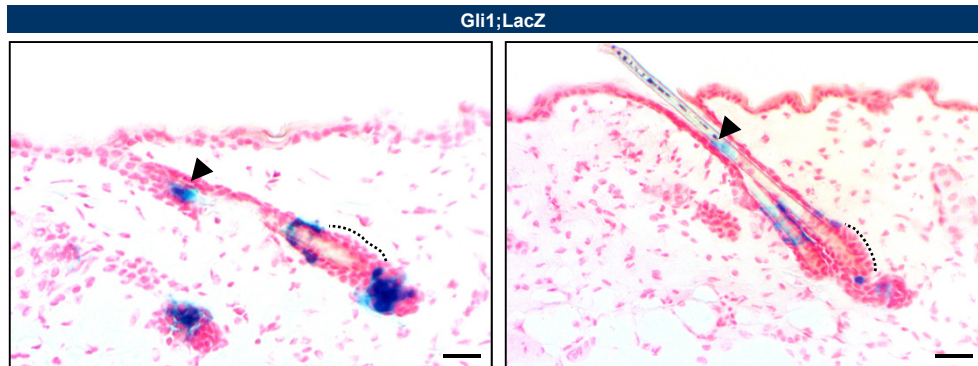


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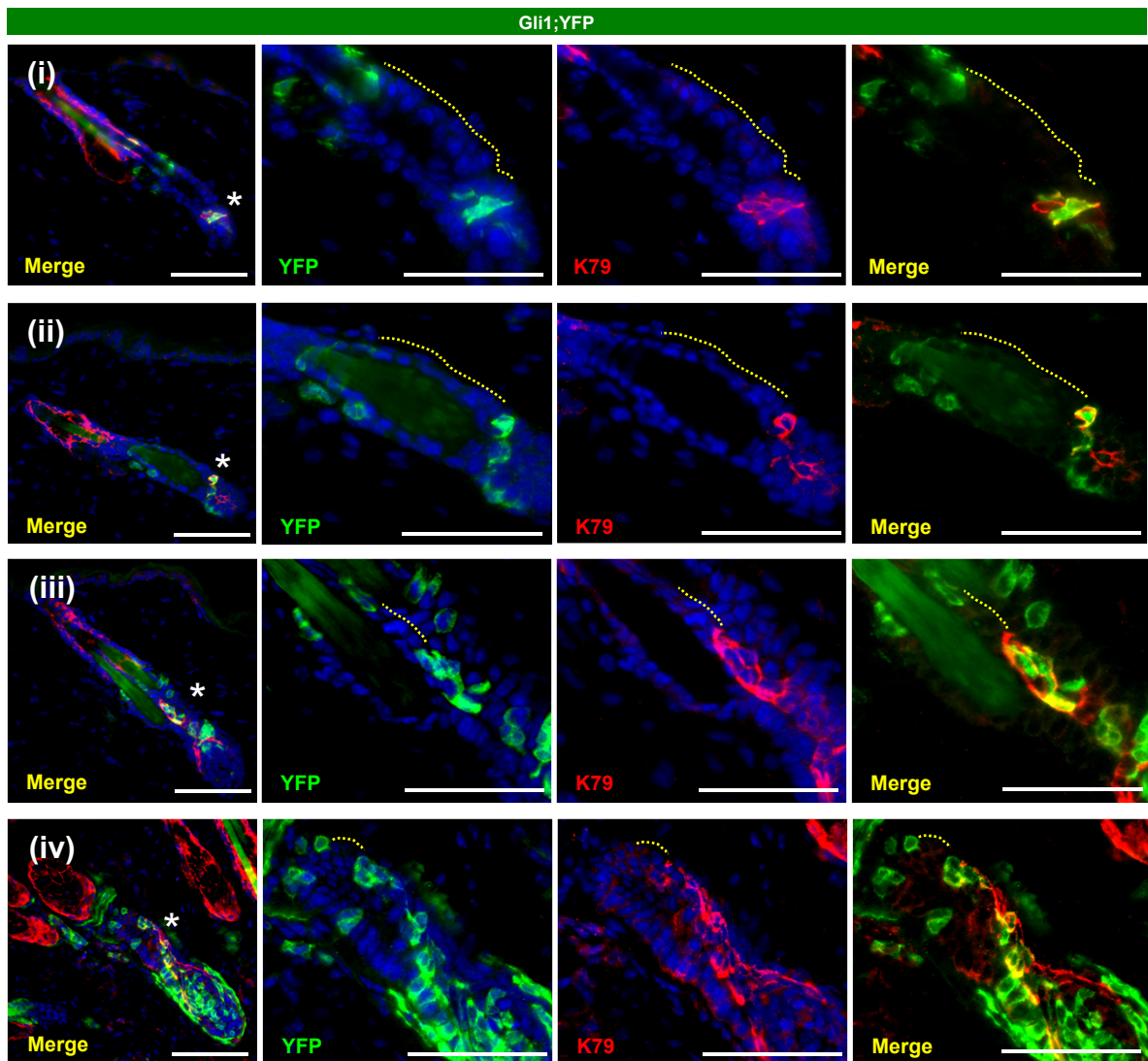


Veniaminova_Fig S11

A



B



Veniaminova_Fig S12

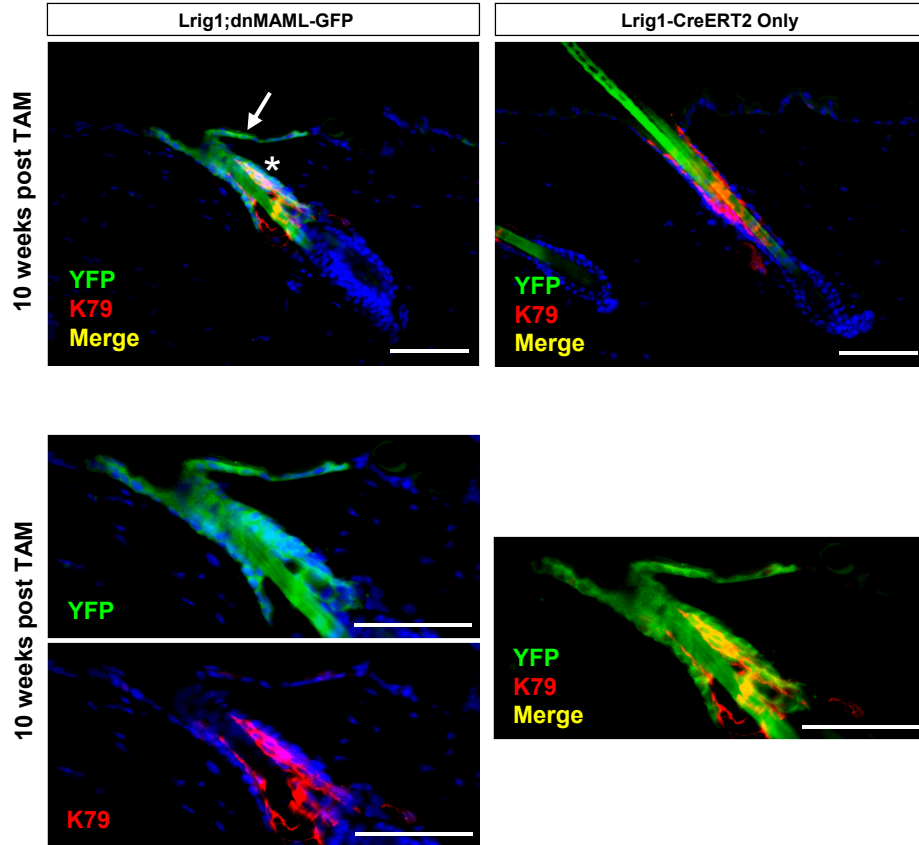


Table S1. Summary of quantitative PCR results in skin.

Results were obtained using either RNA from total skin to determine overall gene expression levels for different keratins, or RNA from flow-sorted cells (suprabasal YFP⁺α6⁻ versus basal YFP⁺α6⁺ cells from *Shh;YFP* mice) to determine whether expression is enriched in suprabasal hair follicle cells. Values shown are gene expression fold change in suprabasal YFP⁺ versus basal YFP⁺ cells. Previous studies have not reported enrichment of K17 in the sINF; however, we observed upregulated expression here in suprabasal cells, concordant with our findings by IHC (Fig. 1C).

Keratin	Type	Candidate protein?	Enriched in suprabasal hair follicle cells?
K1	2	No (Literature)	
K2	2	No (Poorly expressed)	
K4	2	Yes	Yes (2.36)
K5	2	No (Literature)	
K6a	2	No (Literature)	
K6b	2	No (Literature)	
K6c	2	No (Literature)	
K7	2 (S)	No (Literature)	
K8	2 (S)	No (Literature)	
K9	1	No (Poorly expressed)	
K10	1	No (Literature)	
K12	1	No (No enrichment)	No (0.57)
K13	1	No (No enrichment)	No (0.91)
K14	1	No (Literature)	
K15	1	No (Literature)	
K16	1	No (Literature)	
K17	1	No (Literature)	Yes (2.75)
K18	1 (S)	No (Literature)	
K19	1 (S)	No (Literature)	
K20	1 (S)	N/D	
K23	1 (S)	No (Literature)	
K24	1	No (No enrichment)	No (0.03)
K25	1 (IRS)	No (Poorly expressed)	
K26	1 (IRS)	No (Poorly expressed)	
K27	1 (IRS)	No (Poorly expressed)	
K28	1 (IRS)	No (Poorly expressed)	
K31	1 (H)	No (Poorly expressed)	
K32	1 (H)	No (No enrichment)	No (0.62)
K33a	1 (H)	No (Poorly expressed)	
K33b	1 (H)	No (Poorly expressed)	
K34	1 (H)	No (Poorly expressed)	
K35	1 (H)	N/D	
K36	1 (H)	N/D	
K37	1 (H)	No (Not in mouse)	

K38	1 (H)	No (Not in mouse)	
K39	1 (H)	No (Poorly expressed)	
K40	1 (H)	No (Poorly expressed)	
K42	1	No (Poorly expressed)	
K71	2 (IRS)	No (Poorly expressed)	
K72	2 (IRS)	No (Poorly expressed)	
K73	2 (IRS)	No (Poorly expressed)	
K74	2 (IRS)	Yes	Yes (4.9)
K75	2	No (IHC shows CL/inner bulge enriched)	
K76	2	Yes	Yes (2.63)
K77	2	Yes	Yes (1.99)
K78	2	Yes	Yes (3.13)
K79	2	Yes	Yes (20.13)
K80	2	No (No enrichment)	No (0.84)
K81	2 (H)	N/D	
K82	2 (H)	N/D	
K83	2 (H)	N/D	
K84	2 (H)	N/D	
K85	2 (H)	N/D	
K86	2 (H)	N/D	

(S) Simple keratin
(H) Hair keratin
(IRS) Inner root sheath keratin
1 Type 1 keratin
2 Type 2 keratin
'Literature' Localization well-characterized in previous studies; unlikely to be Ag-7195
'Poorly expressed' Poor overall amplification by qPCR on RNA from total skin RNA
'No enrichment' Gene candidate not enriched in purified suprabasal hair follicle cells
'N/D' Not determined

Table S2. Summary of IHC results on human acne samples.

Patient #	Gender	Age	Lumen Area (Pixels)	K79	K5	K10	K17
64	F	37	531,600	-	+	+	+
65	F	24	63,428	+	+	+	+
66	F	22	187,979	+	+	+	+
68	M	21	580,004	-/+	+	+	+
72	F	22	86,626	-	+	+	N/D
74	F	24	618,750	-	+	+	+
75	F	24	967,547	-	+	+	N/D

Table S3. Primer sequences for quantitative PCR.

Name	Forward	Reverse
Hprt	AGGACCTCTCGAAGTGTTGGATAC	AACTTGCGCTCATCTTAGGCTTTG
Itga6	AGACCAGTGGATGGGAGTCA	ACGTGCTGCCGTTTCTCATA
K2	CTGTCCCTGGATGTGGAGAT	CCGAAGCCAGTCTTAGATGC
K14	CGCCGCCCCCTGGTGTGG	ATCTGGCGGTTGGTGGAGGTCA
K10	GGAGGGTAAAATCAAGGAGTGGTA	TCAATCTGCAGCAGCACGTT
K4	GAGCCTGCTGACACCTCTTC	GATGAAGGACGCGAATTTGT
K9	CCATCTCAGTCCCAGTCCTC	TCCGGTGGAGAAAGTGAATC
K12	AGCTCCTCCTGCAGATTGAC	AGGGCCAGCTCATTCTCAT
K13	CCGAAGTGAGATGGAGTGCC	GGACCCGTTGGAGGTAGTAG
K17	ACCATGCAGGCCCTGGAGA	GTCTTCACATCCAGCAGGA
K24	GGAAGAGACTACAGCCAATAC	CTCGAAGGCAGAGTTCATGCT
K25	GAGCGAGGAGCTGACCTATC	TGTTGTTTACGACGAGGACGGTG
K26	CAGCAGATCCGAACAGAGACGG	AGGCTTGCCATCCTTTGGTTTG
K27	AGCAGCAGATTTTCAGACGATGC	CAGTAGTTGCCCTCGGTCTCC
K28	AGACCTACTGCCGCCTCATA	CCGTCTTACCAGTGTGGTT
K31	AAGCTACCTTGCAACCCCTG	ATCCCCCAGGTTCTAGCGTA
K32	CCTGCTGGAGAGTGAGGACA	AAGGCACACAAACAGTGTGG
K33a	ACCAGGCCTACTTCAGGACC	ACCAGCTGCCGCAGACTAAG
K33b	CAAGAGAGGAACCAGCAGCA	CCGTTCTCAGACTTGCCACA
K34	TGGAGTCTCTGAGGGAGGAG	ACCTGGTCTCGTTGAGCACT
K39	GATAGCCACATACCGAAGCC	GTGAATCCCAGGGAGTGATG
K40	ATGTTGGAGTTGAAGCGCAAG	ATTAGGCACTGCATTTGGGC
K42	GCGTTGTGACATGGAAAGGC	GCCAGGGATGAGGAGTATTG
K71	GGACCCTGAGATCCAGAAGG	AGCAGCTCCCCTTGGTCT
K72	GCTGAGGAACATGAGGGAAG	CACATCCTTCTTCAGCACCA
K73	GAGGACATTGCCCTGAAGAG	TGGTGTGCTTGAGGTCATCT
K74	GATCCGCTGTGACATTGCTA	CCTCCAGCTCATCCAGCTT
K76	TCATGAATGTCAAGCTGGCC	TTGCTGACCACTGAAATGC
K77	ATGTTCTGACCACCGAGCAGT	GATTTGCCCATGAAAGCAGCA
K78	AGGCTGCAGAGTCAGATTGG	ATCCCTCACAGCCATCTCC
K79	TTGACTTCCTGCAACAGCTC	GATGCTGTCCAAGTCCAGGT
K80	CGTAAAGTGAGCCAAGAGCG	CGCTTGAGATCTCGTCTCTC
Gli2-5'	CCTTCTAATGCAGAGCGGGG	TCATGCGTTGTAGGTTCGAGG
Gli2-3'	GGTGGTACCTTGGATGACGG	AGCAGAAGGACCCATGTTGG

Two *Gli2* primer sets are shown, designed to amplify either 5' or 3' regions of the mRNA.