

Expanded View Figures

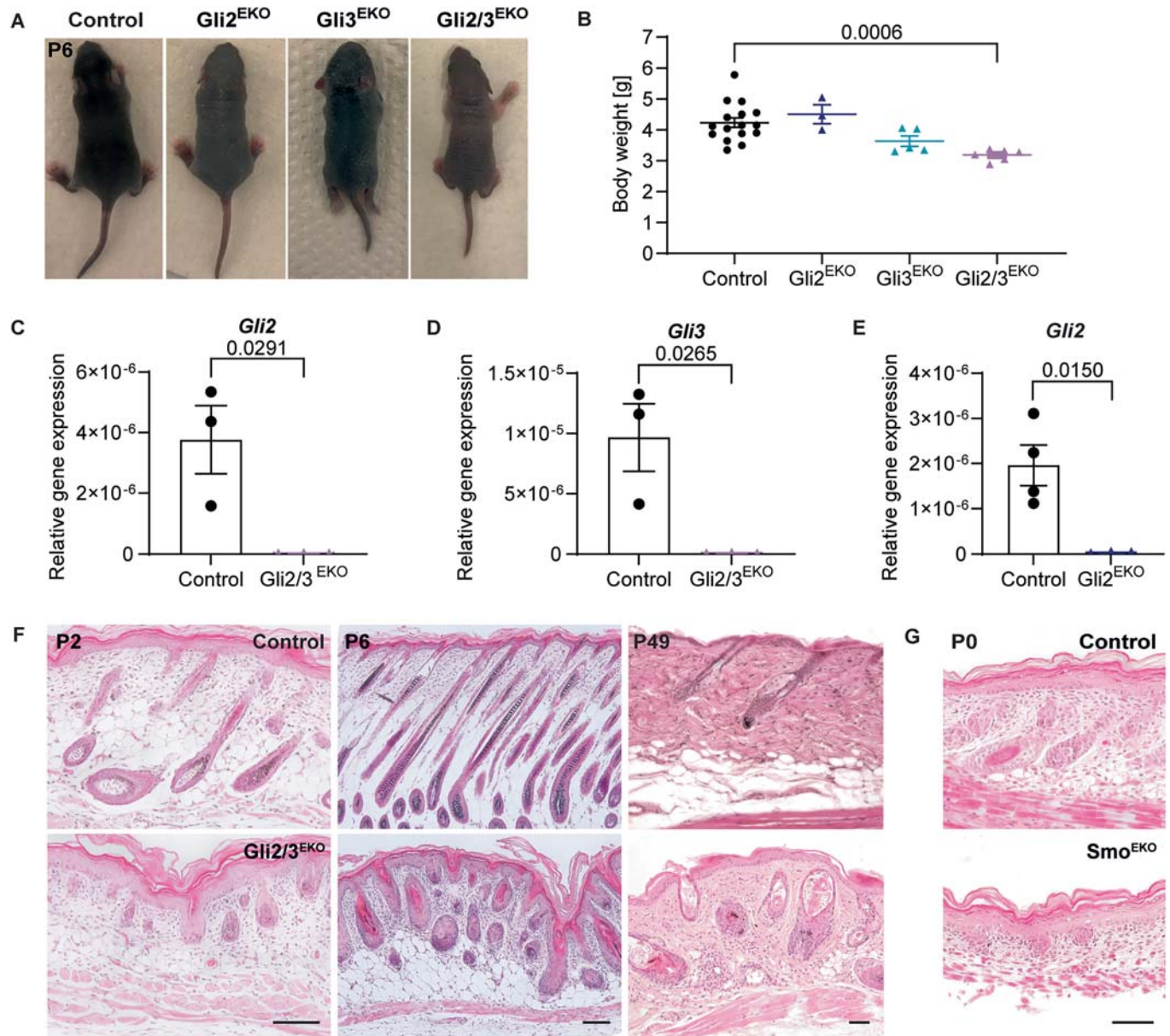


Figure EV1. Characterisation of Gli^{EKO} and Smo^{EKO} mouse models.

(A, B) Representative images (A) and body weight (B) of P6 Gli2^{EKO}, Gli3^{EKO}, Gli2/3^{EKO} and control mice. ($n = 3-16$ mice/genotype). (C, D) qRT-PCR analysis for Gli2 (C) and Gli3 (D) mRNA expression in tail epidermis from P6 Gli2/3^{EKO} and control littermates. Each datapoint represents one animal. ($n = 3$ mice/genotype). (E) qRT-PCR analysis for Gli2 mRNA expression in back skin epidermis from P3 Gli2^{EKO} and control littermates. Each datapoint represents one animal. ($n = 3-4$ mice/genotype). (F) Representative H&E staining of back skin sections from P2, P6 and P49 Gli2/3^{EKO} and control littermates ($n = 3$ mice/genotype). (G) Representative H&E staining of back skin sections from P0 Smo^{EKO} and control littermates. Each datapoint represents one animal. ($n = 3$ mice/genotype). Scale bars 50 μ m (F, G). Data were presented as mean $\pm$ SEM. P value was calculated using an unpaired Student's t -test.

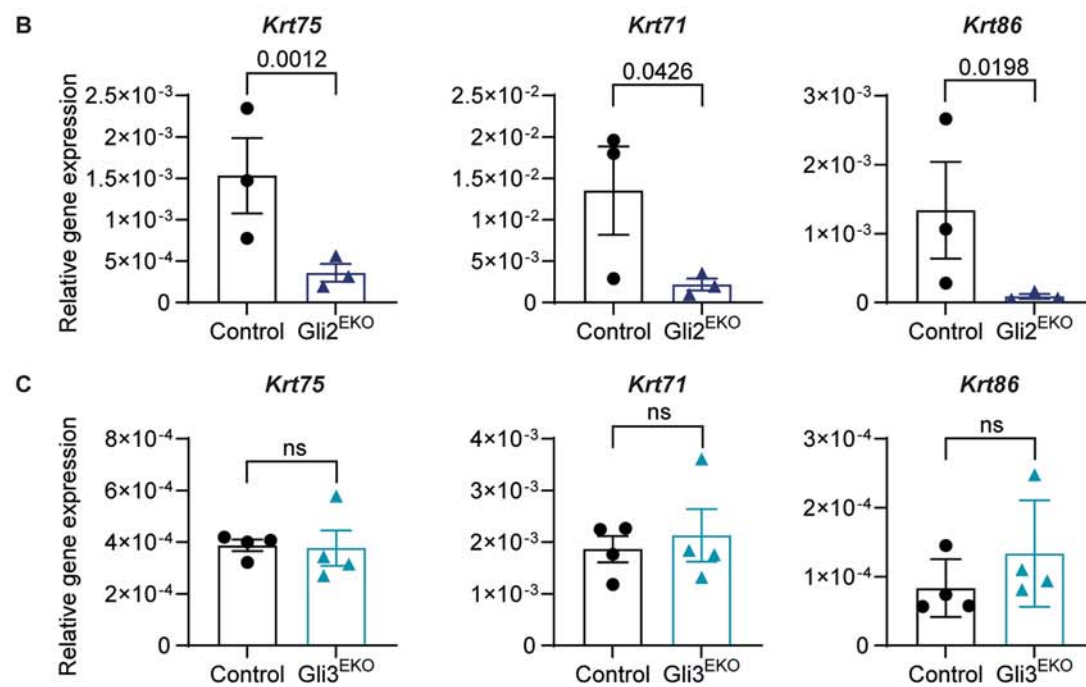
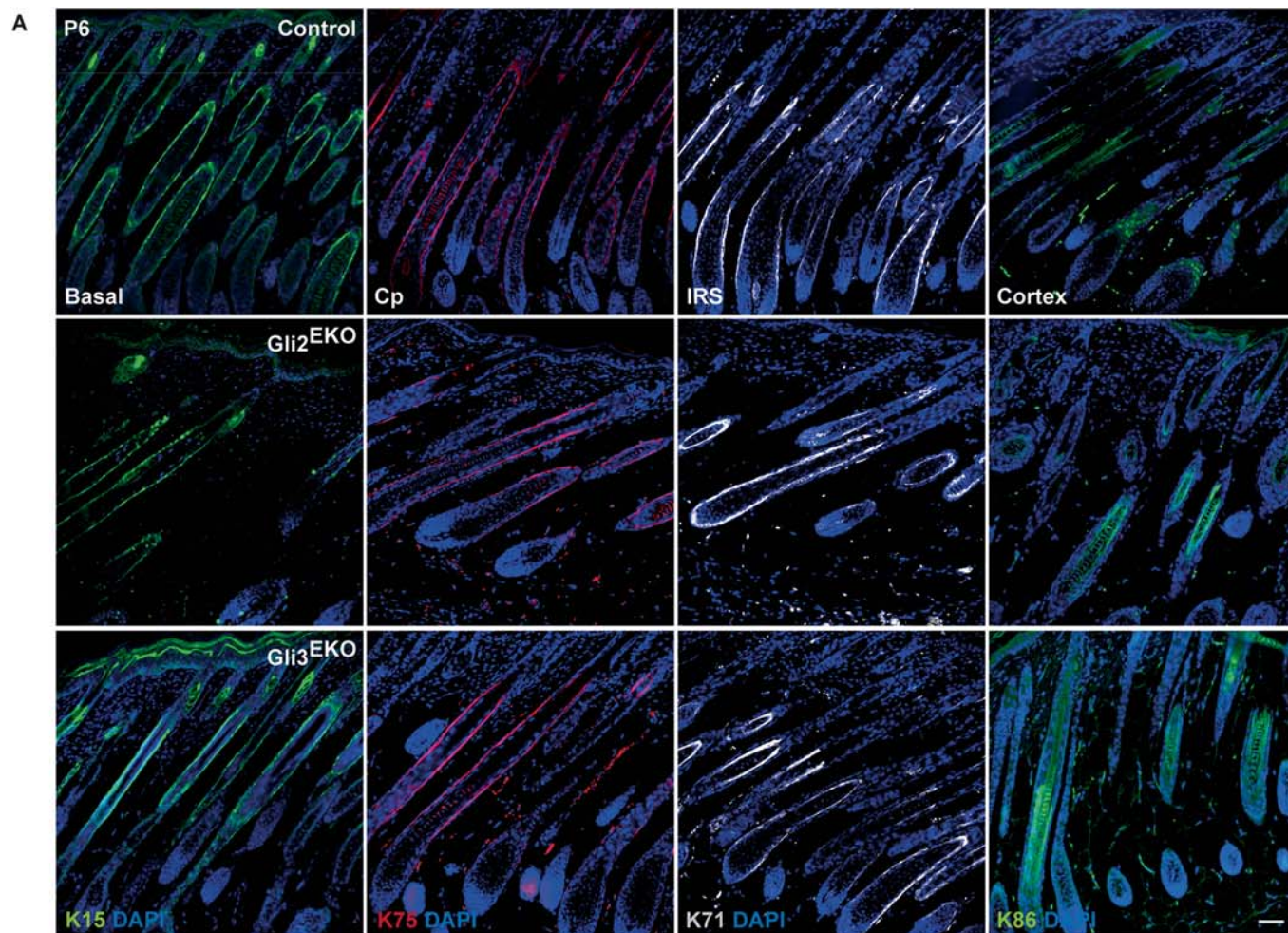
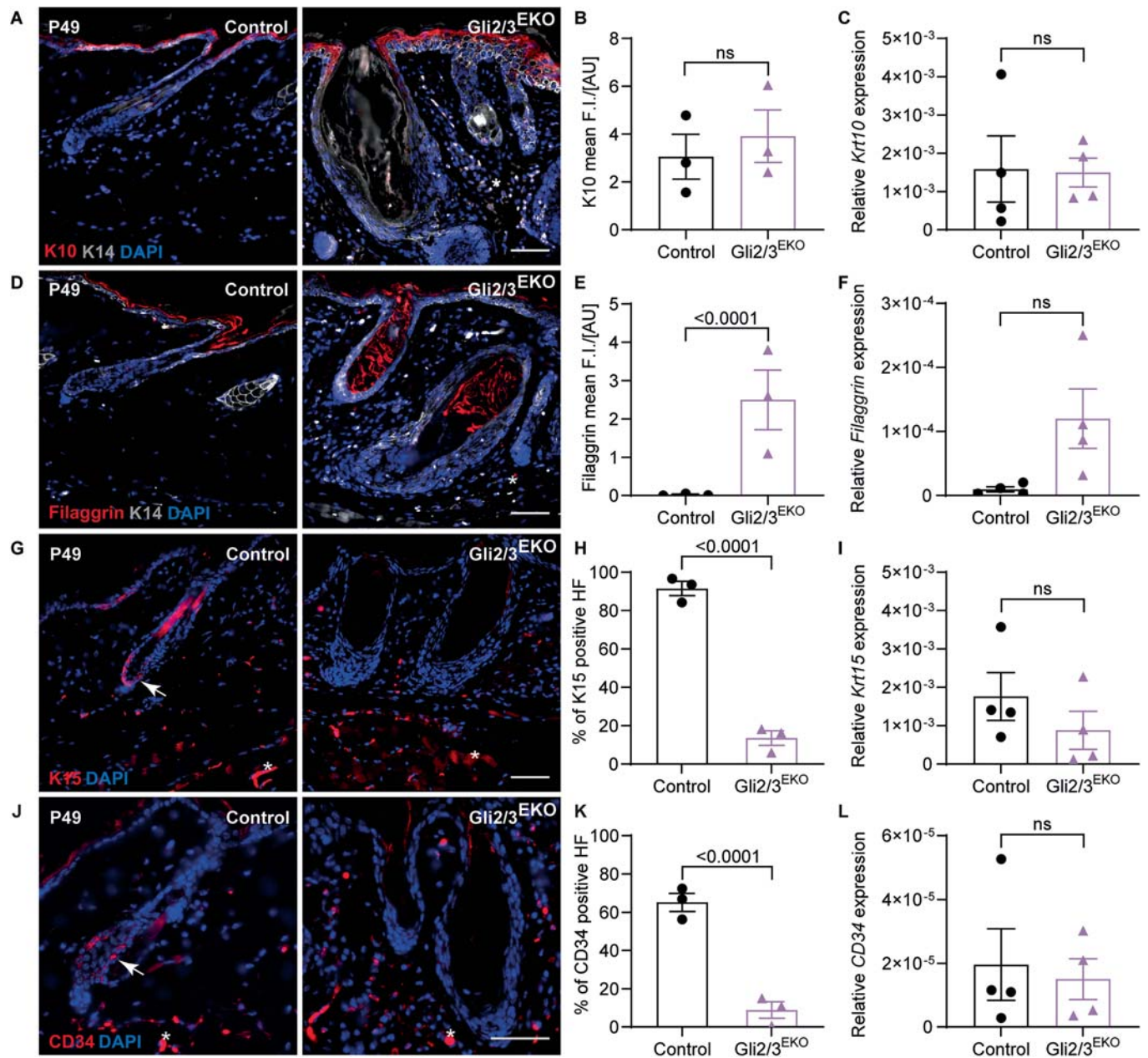


Figure EV2. Analysis of hair lineage differentiation in Gli2^{EKO} and Gli3^{EKO} mice.

(A) Immunofluorescence staining for K15 (green), K75 (red), K71 (grey), K86 (green) and DAPI (blue, nuclei) of back skin sections from P6 Gli2^{EKO}, Gli3^{EKO} and control mice. ($n = 3$ mice/genotype) (redisplay of data for control mice from Fig. 2A). (B, C) qRT-PCR analysis of Krt75, Krt71 and Krt86 mRNA expression in back skin of P6 Gli2^{EKO} (B) and Gli3^{EKO} (C) and control littermates. ($n = 3$ –4 mice/genotype). Scale bar 50 μ m. Data were represented as mean $\pm$ SEM. P value was calculated using paired (B) and unpaired Student's t -test (C).



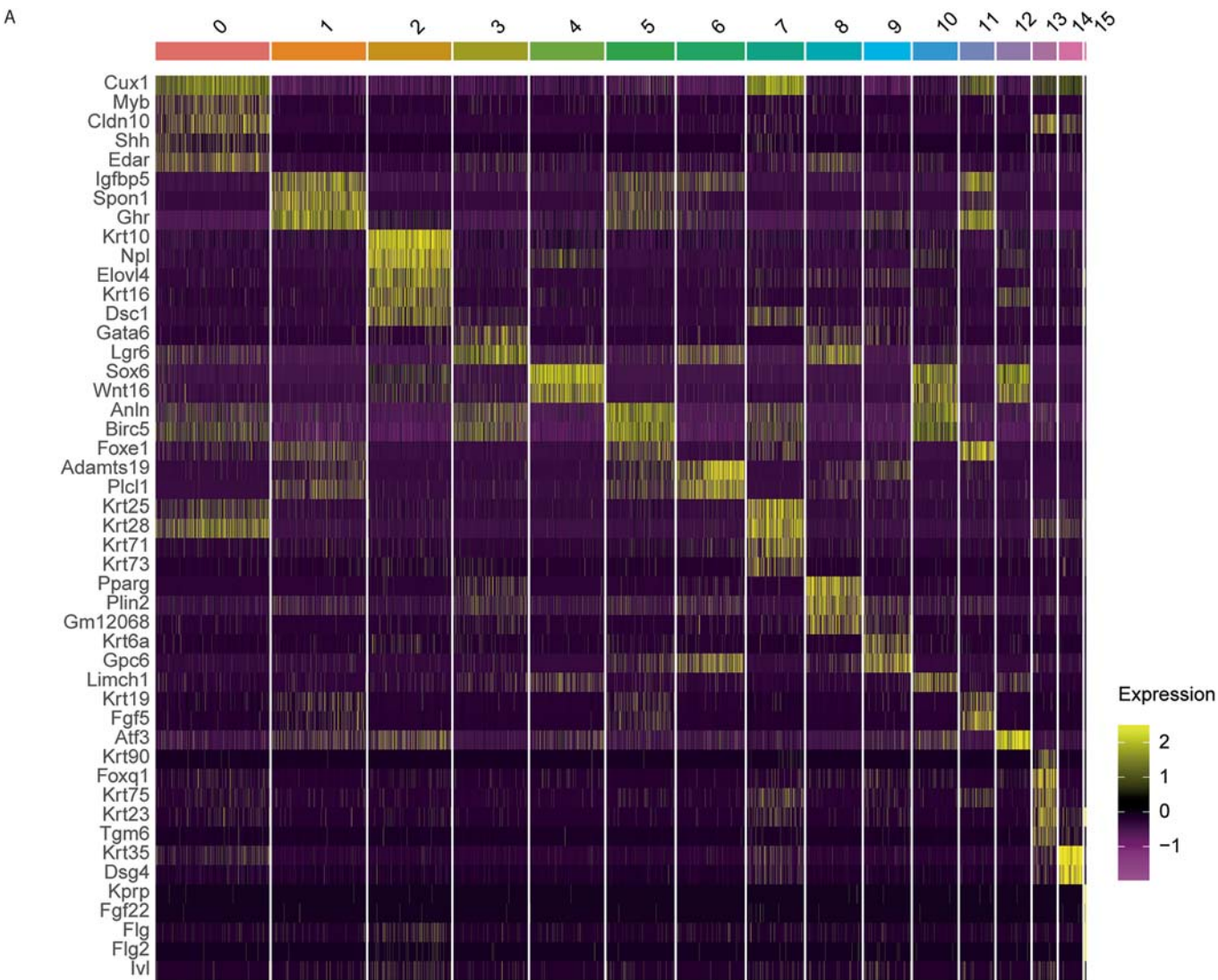


Figure EV4. Marker expression in keratinocyte clusters.

(A) Heatmap showing the marker expression defining each cluster. Colours represent scaled expression by gene.

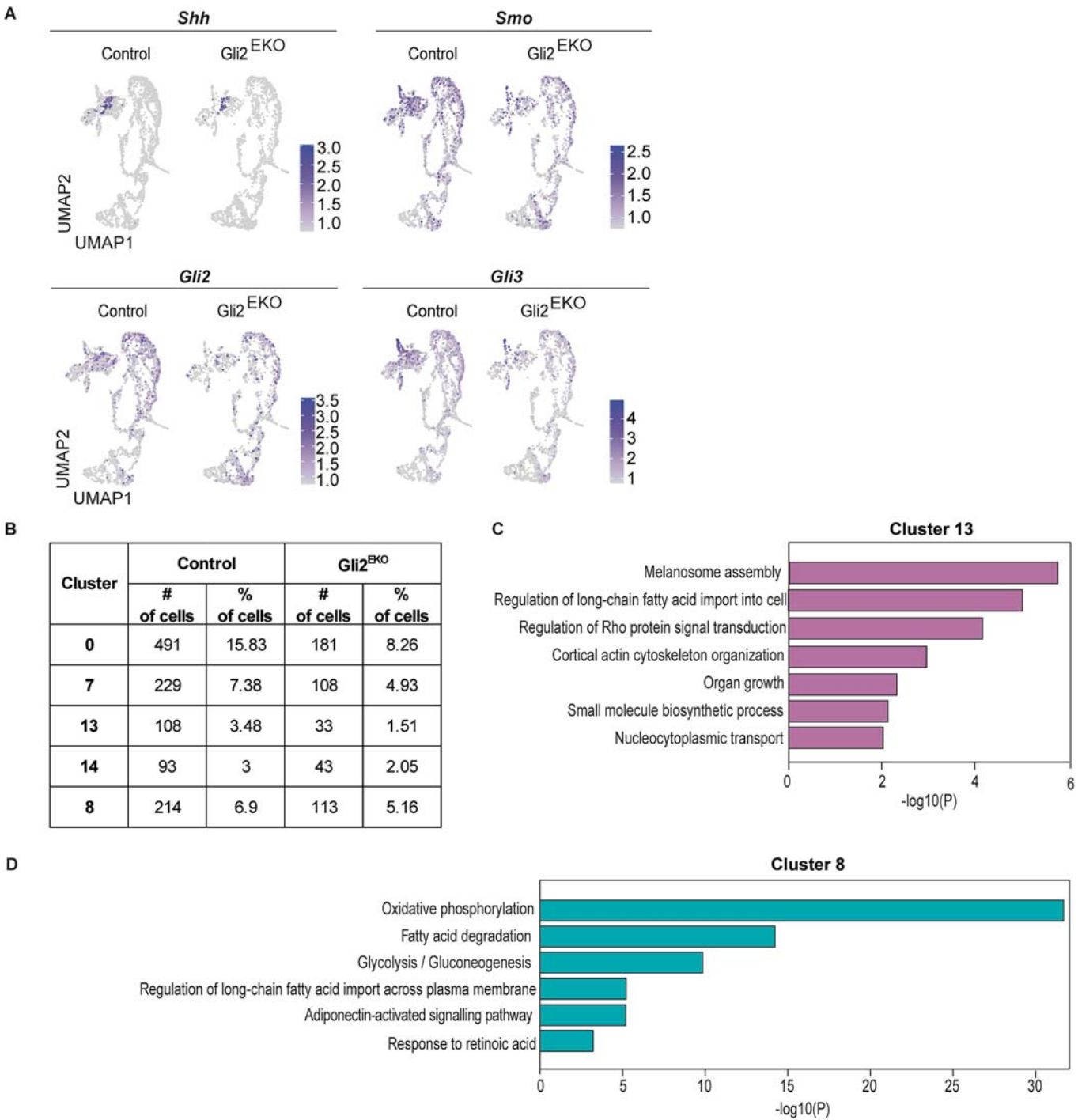


Figure EV5. Molecular and cellular characterisation of distinct keratinocyte populations in *Gli2*^{EKO} mice.

(A) Feature plots of scRNAseq data showing expression of *Shh*, *Smo*, *Gli2*, and *Gli3* in Control vs *Gli2*^{EKO} keratinocytes. Expression levels are colour coded, and expression is shown for values greater than 0.5. (B) Numbers and percentages of keratinocytes for clusters 0, 7, 13, 14 and 8 in control vs. *Gli2*^{EKO} scRNAseq dataset. (C, D) Gene ontology for pathway and process enrichment analysis of the top 110–130 down- and up-regulated genes in clusters 13 (C) and 8 (D), respectively. *P* values were calculated based on the cumulative hypergeometric distribution.