Cell Reports, Volume *42*

Supplemental information

Distinct mechanisms for sebaceous gland self-renewal and regeneration provide durability in response to injury

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Veniaminova, Jia_Fig S1

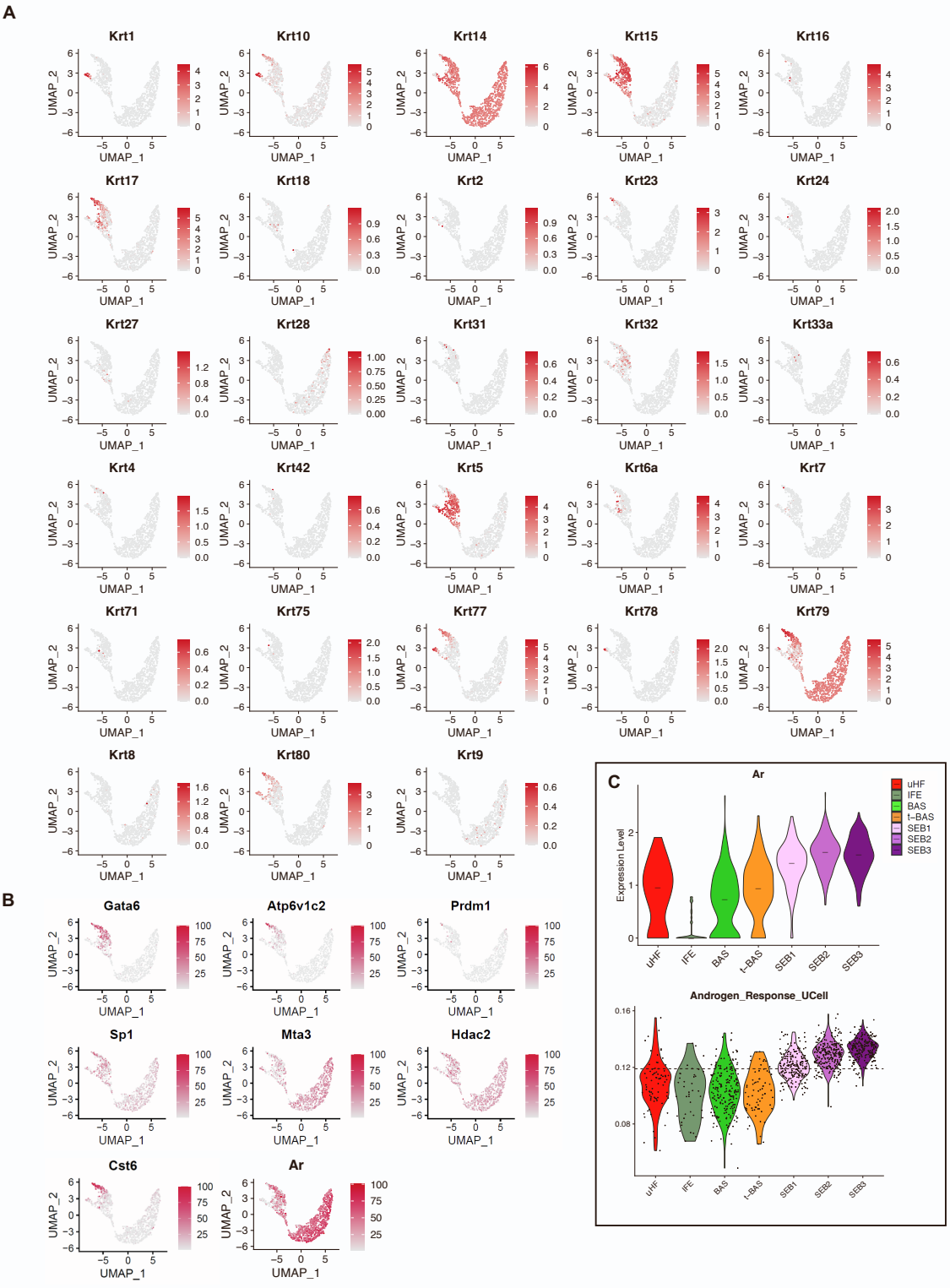

Figure S1. Keratin gene expression and androgen response in the SG. A. Feature plots for 28 keratin genes. Only keratin genes that met filtering criteria are depicted. **B.** Feature plots for androgen receptor (AR) and upper hair follicle markers described by Donati et al.,[1] and others. **C.** Top, violin plot for AR. Bottom, violin plot for "hallmark androgen response" gene set from MSigDB (96 genes) plus 3 additional genes associated with AR response, previously reported by Cottle et al.[2] (*Myc*, *Ar*, *Trp53*). Dashed line indicates the mean score across all cells. Related to Figure 3.

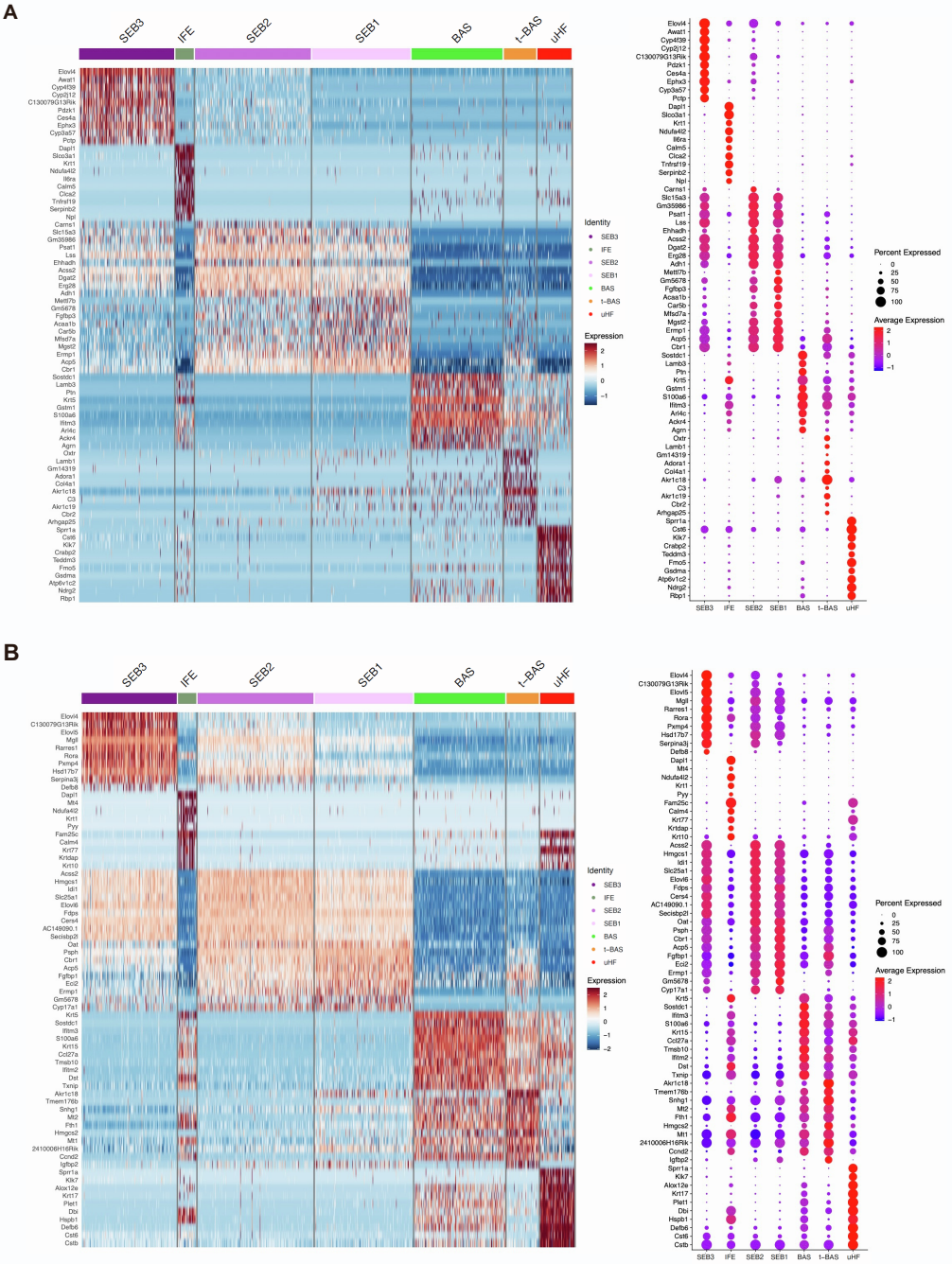

Figure S2. Top 10 genes enriched in each cell cluster. Data were calculated by COSG (**A**) or Seurat-Wilcoxon (**B**), depicted by heat maps (left) or dot plots (right). Red denotes high expression, blue denotes low expression. Related to Figure 3.

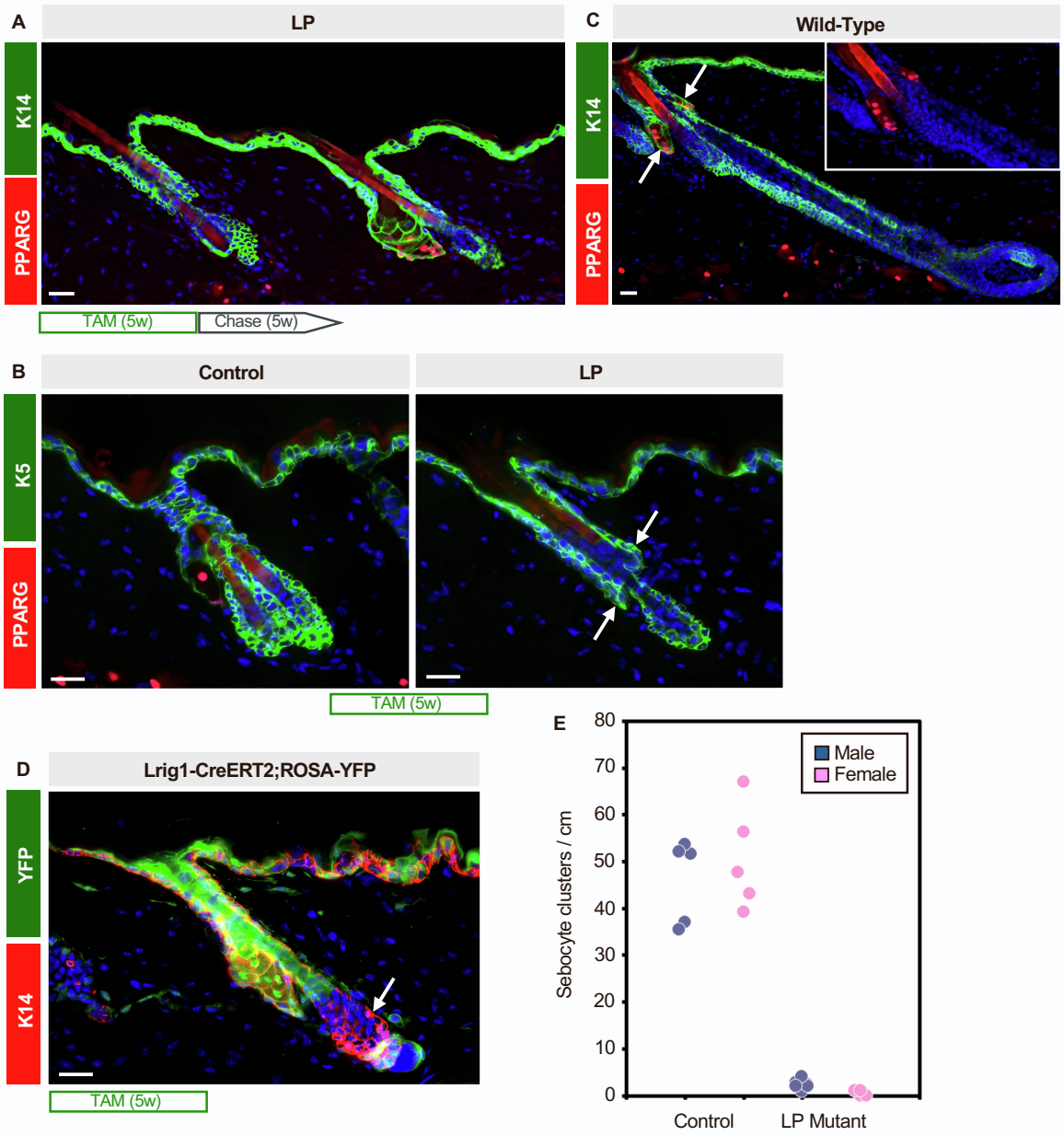

Figure S3. PPARγ expression and its deletion by Lrig1-CreERT2-mediated recombination. A. Regenerated SG (right hair follicle) has PPARγ+ cells (red), after 5 weeks of TAM chow treatment and 5 weeks' chase. **B.** TAM-treated, mutant LP hair follicles possess K5+PPARγ-negative "nubs" at sites where SGs would normally be located (arrows). **C.** PPARγ (red) is expressed in SGs (arrows), but not in bulge or ORS cells in wild-type anagen hair follicles. Inset shows a magnified, single channel view of the bulge and upper ORS. **D.** Lrig1-CreERT2 does not induce recombination, as assessed by YFP reporter expression (green), in the hair follicle bulge (arrow), following 5 weeks of TAM chow treatment. **E.** Quantitation of SG abundance in LP mice treated for 2 weeks with TAM chow beginning at 6 weeks of age. SGs were counted in frozen skin sections stained for PPARγ and Scd1. Control animals were Cre-negative littermates treated with TAM chow. n = 10 mice per genotype. Scale bar, 50 μm. Related to Figure 6.

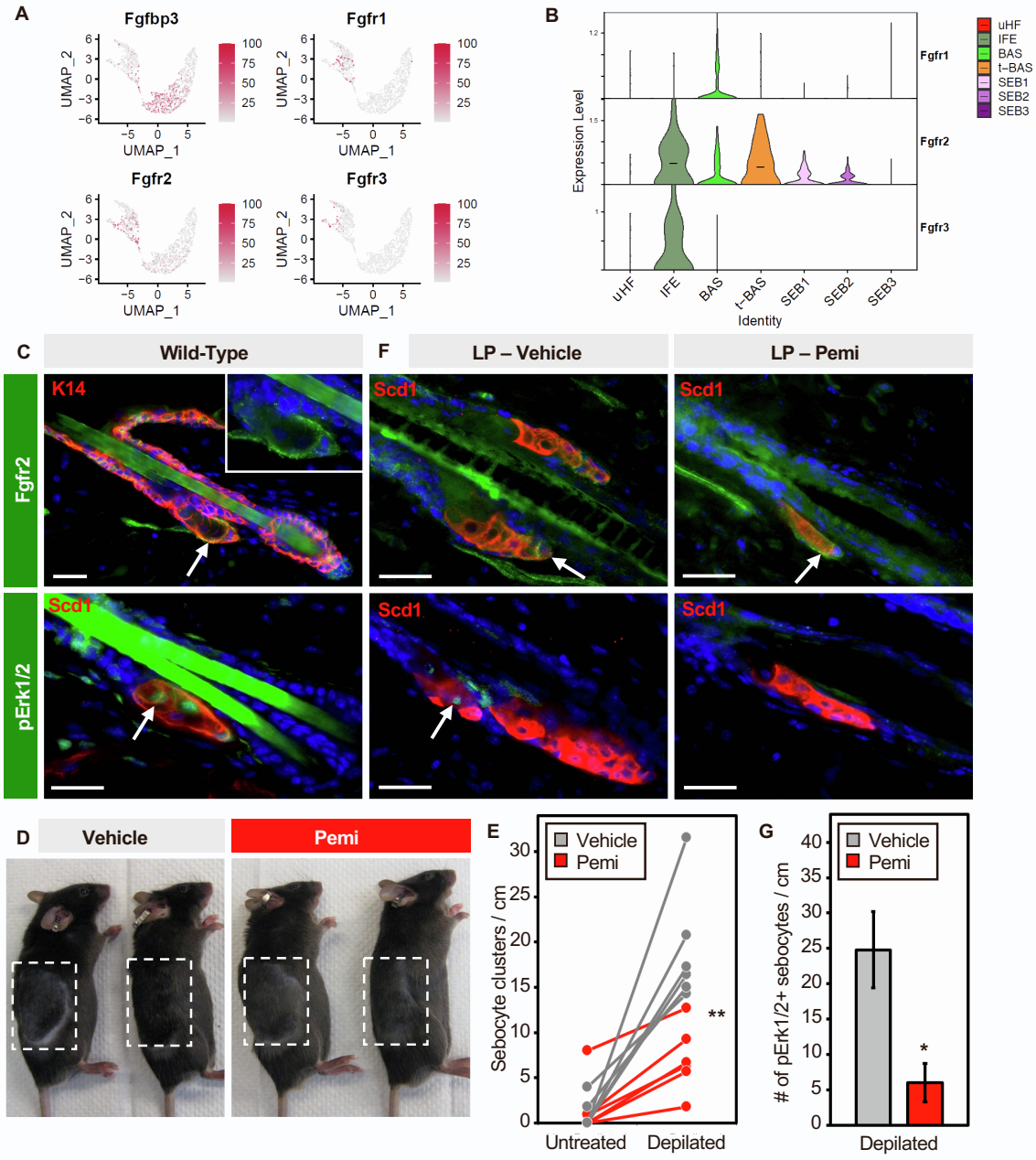

Figure S4. FGF signaling during SG homeostasis and regeneration. A. Feature plots for *Fgfbp3* and *Fgfr1-3*. *Fgfr4* is not expressed in our data-set (not shown). **B.** Violin plots for *Fgfr1-3*. **C.** Localization of FGFR2 (top, green) or phosphorylated-p44/42 (pErk1/2) (bottom, green) in SGs from wild-type telogen hair follicles (arrows). **D.** Skin from LP mice that were depilated (boxed areas) and treated systemically with vehicle or pemi during the 2 week chase entered anagen and regrew hair normally. **E.** SG quantitation in LP mice treated with 2 weeks of TAM chow beginning at 6 weeks of age, then depilated, and treated with either vehicle (grey) or pemi (red) during the 2 week chase period. Paired samples collected from the same mouse are connected by lines. Quantitation was performed on the same animals as shown in Figure 7H, using an alternative quantitation method where SGs were counted from H&E sections. n = 11 mice. **F.** Pemi-treated skin did not exhibit obvious changes in overall FGFR2 levels or localization in SGs (top panels, arrows), but had reduced pErk1/2+ sebocytes (bottom panels, arrows). **G.** Quantitation of pErk1/2+ sebocytes in vehicle or pemi-treated LP mice, depilated skin only. n = 4 mice per treatment group. Data are represented as mean +/- SEM. *, $p < 0.05$ by unpaired t-test. **, $p < 0.01$ by unpaired t-test comparing depilated pemi-treated versus depilated vehicle-treated samples. Scale bar, 50 µm. Related to Figure 7.

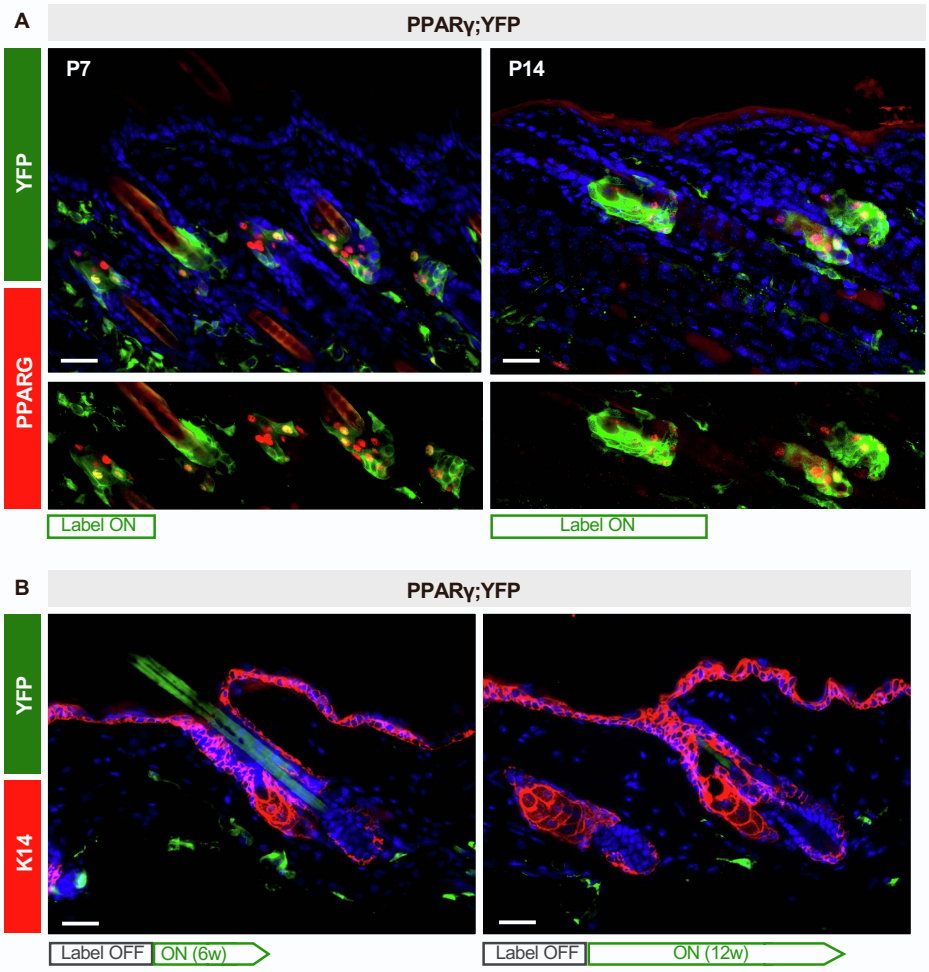

Figure S5. Reporter gene expression in PPARγ;YFP mice. A. Skin from PPARγ;YFP label-on mice, harvested at 1 week (left) or 2 weeks (right) of age. Note the heterogeneous recombination, as assessed by YFP reporter expression (green), in PPARγ+ cells (red) as well as in PPARγ-negative cells. Lower panels show identical views as above, with DAPI omitted. **B.** Skin from PPARγ;YFP mice that were treated with doxy since gestation until 8 weeks of age (label-off), then followed for an additional 6 weeks (left) or 12 weeks (right) after doxy removal (label-off→on). Note that this approach does not cause YFP reporter expression in this system. Scale bar, 50 μm. Related to Figure 7.

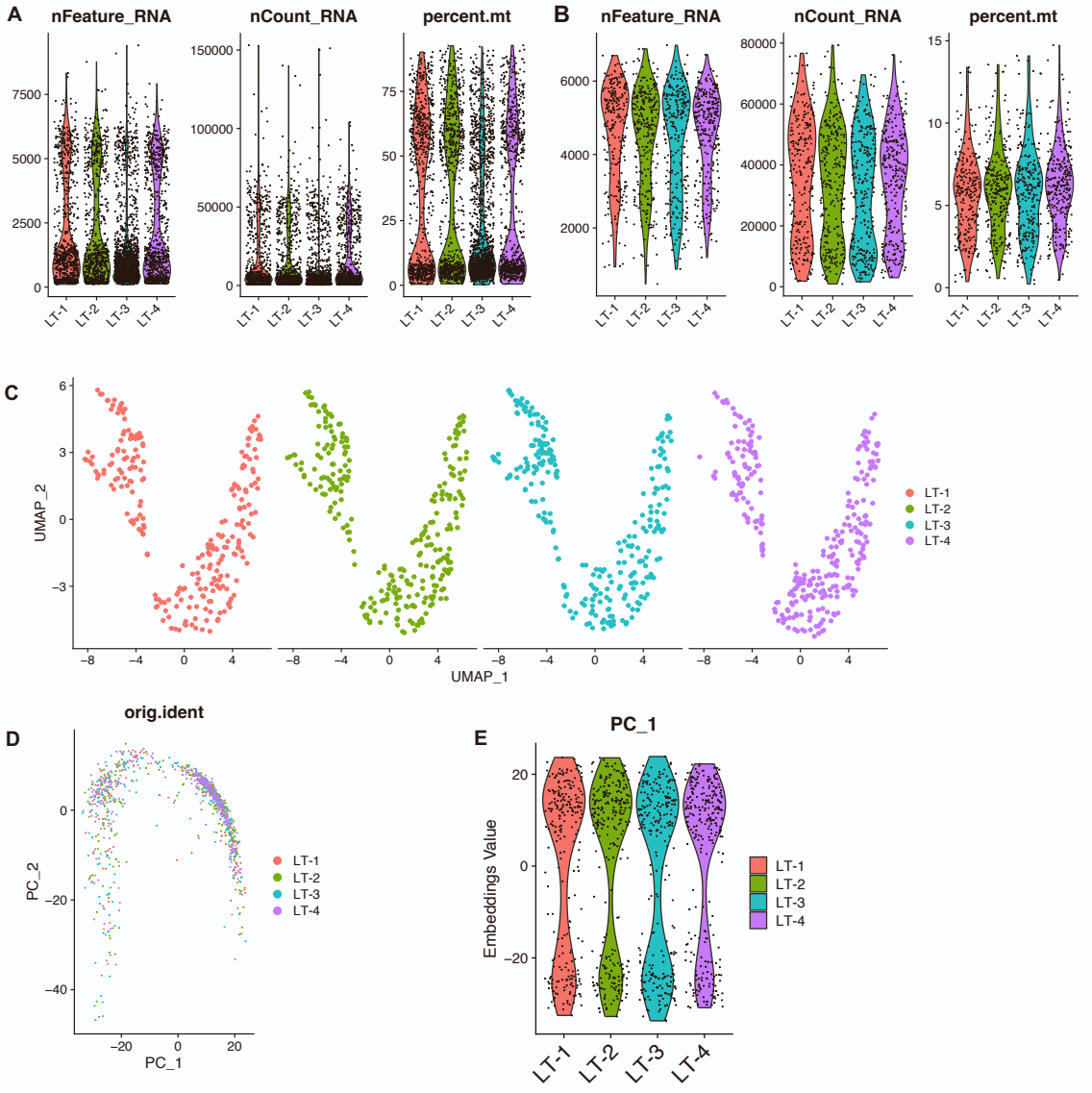

Figure S6. Metrics for single cell libraries. A. Quality control metrics for 4 replicate single cell libraries (LT1-4) before threshold filtering. **B.** Metrics of single cell libraries after threshold filtering. Cells with <200 detected genes as well as those with >15% mitochondrial content were removed. Cells having >7,000 detected genes were removed to exclude potential doublets. Cells with <900 and >80,000 UMIs per cell were removed. Genes detected in <3 cells were removed. **C.** Each library was projected by UMAP and found not to exhibit significant batch effects. **D.** PCA analysis combining all 4 replicates. **E.** PC1 score distribution by VlnPlot across 4 replicates. Related to STAR Methods.

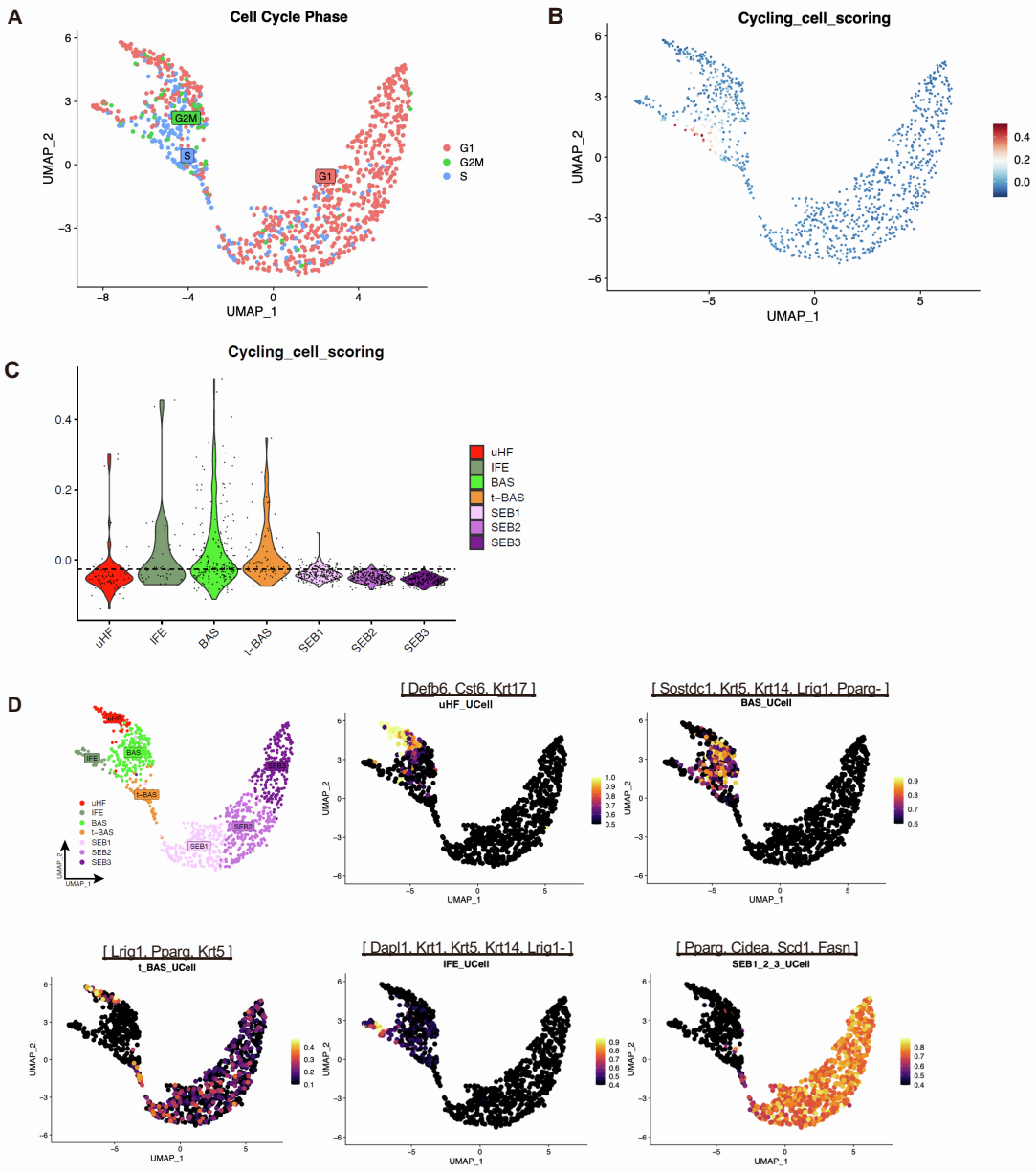

Figure S7. Cell cycle and cell type scoring. A. Cell cycle annotation performed using the Cell Cycle Scoring function in Seurat. **B.** Cycling cell scoring. A set of S- and G2M-phase genes was combined and used for cycling cell scoring, which was calculated by the AddModuleScore function with default parameters in Seurat. **C.** Cycling cell scoring across different cell states. Dashed line indicates the mean score across all cells. **D.** Cell type signature scoring calculated by UCell to identify cell populations (see Methods for details). Key genes for each cell type used in the signature are shown. Related to STAR Methods.

References

1. Donati, G., Rognoni, E., Hiratsuka, T., Liakath-Ali, K., Hoste, E., Kar, G., Kayikci, M., Russell, R., Kretzschmar, K., Mulder, K.W., et al. (2017). Wounding induces dedifferentiation of epidermal Gata6+ cells and acquisition of stem cell properties. Nat Cell Biol 19, 603-613.

2. Cottle, D.L., Kretzschmar, K., Schweiger, P.J., Quist, S.R., Gollnick, H.P., Natsuga, K., Aoyagi, S., and Watt, F.M. (2013). c-MYC-induced sebaceous gland differentiation is controlled by an androgen receptor/p53 axis. Cell Rep 3, 427-441.