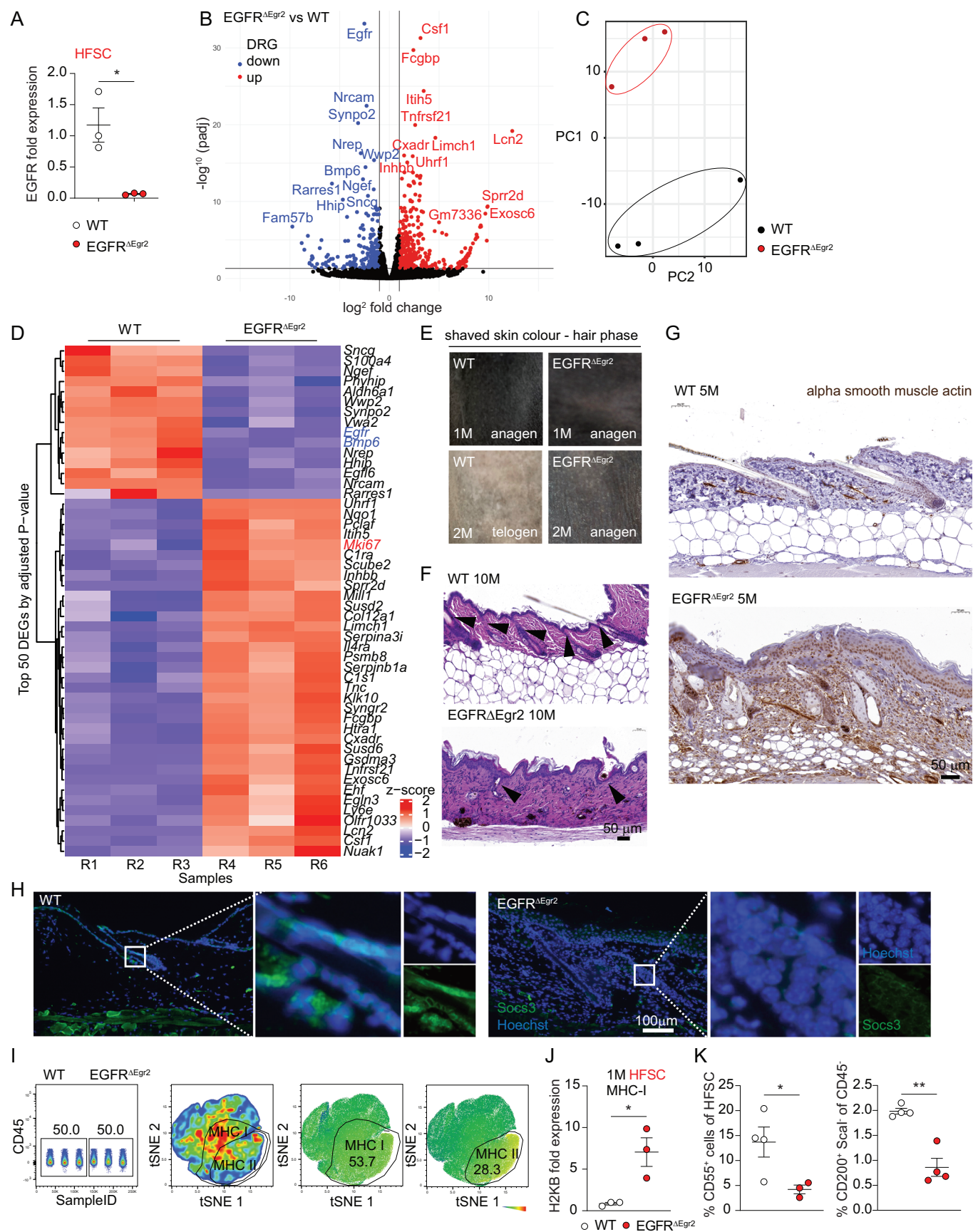


Expanded View Figures

Figure EV1. Hair follicle-specific EGFR deletion induces epidermal immune infiltrate and microbiota-driven inflammatory hair follicle destruction.

(A) Representative pictures of the gating strategy of FACS analysis of epidermal single cell suspensions (WT and EGFR^{ΔEgr2} mice). CD34⁺ Sca-1⁻ HFSC, CD34⁺ Sca-1⁻ HF and Sca-1⁺ IFE among CD45⁺ keratinocytes. γδTCR^{hi} dendritic epidermal T cells (DETC), γδTCR^{int} γδT cells (γδTC), αβT cells (αβTC), CD11b⁺Gr-1⁺ neutrophils, NK1.1⁺ Nkp46⁺ natural killer (NK) cells, CD11b⁺Epcam⁺ Langerhans cells (LC) among CD45⁺ immune cells. (B) FACS analysis of αβT cells (WT vs EGFR^{Δep} $p = 0.0067$, EGFR^{Δep} vs WT Abx $p = 0.001$, EGFR^{Δep} vs EGFR^{Δep} K5-SOS Abx $p = 0.0057$) and γδT cells (WT vs EGFR^{Δep} K5-SOS $p < 0.0001$, EGFR^{Δep} vs EGFR^{Δep} K5-SOS $p = 0.013$, EGFR^{Δep} K5-SOS vs WT Abx $p < 0.0001$, EGFR^{Δep} K5-SOS vs EGFR^{Δep} Abx $p = 0.0026$, EGFR^{Δep} K5-SOS vs EGFR^{Δep} K5-SOS Abx $p = 0.0004$) among CD45⁺ immune cells at 2 M. Each dot represents an independent mouse. Mouse models as indicated in the graph. (C) Kaplan-Meier survival plot of EGFR^{ΔEgr2} mice or WT. (D) Quantification of EGFR immunohistochemistry staining by Definiens software. Percentage of EGFR expression in hair follicle ($p = 0.0165$) and epidermis of EGFR^{ΔEgr2} mice or WT at 3 M. (E) Representative pictures of hair follicle length measured from hematoxylin and eosin (H&E) stained skin sections marked in yellow dotted lines of WT or EGFR^{ΔEgr2}. (F) Sox9 (red) and CD34 (green) positive stem cells of WT and EGFR^{ΔEgr2} mice at 2, 3, and 5 months. Quantified by Halo AI software in percentage of the hair follicle (CD34⁺ $p = 0.0227$, Sox9⁺ $p = 0.0055$). (G) Timeline P8-P150 showing representative pictures of H&E stainings of WT and EGFR^{ΔEgr2}. Data is presented in $\pm$ SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ by One-Way ANOVA with Tukey's posthoc correction, $n \geq 3$.





◀ **Figure EV2. RNA profiling of FACS sorted EGFR-deficient hair follicle stem cells before scarring hair follicle destruction.**

(A) Real-time PCR of EGFR expression of sorted CD34⁺Scal⁺ HFSCs of WT and EGFR^{ΔEgr2} mice at 1 M. Data is presented in $\pm$ SEM, $p = 0.0156$ by unpaired t-test, $n \geq 3$. (B) Volcano plot of differentially expressed genes (blue downregulated, red upregulated) of RNA sequencing analysis of CD34⁺ HFSCs from WT vs EGFR^{ΔEgr2} mice. Data shown as fold change ($\log_2$) and p -value ($-\log_{10}$). DESeq2 including local dispersion estimation and Independent Hypothesis Weighting (IHW) (x) to estimate false discovery rates and power maximization were used for statistical analysis (Ignatiadis et al, 2016). (C) Principal component (PC) analysis of the RNAseq dataset. (D) Heatmap of z-scores of top 50 differentially expressed genes by adjusted p -value of the RNAseq dataset. Genes of special interest are marked in red (up) or blue (down). Statistics DESeq2 as in (B). (E) Pictures of shaved backs of WT or EGFR^{ΔEgr2} at 1 M and 2 M of age. (F) Representative H&E stained skin sections for counting the number of hair follicle units per 1500 μ m of WT and EGFR^{ΔEgr2} skin at 10 M of age. Black arrowheads indicate counted hair follicles. (G) Representative alpha smooth muscle actin staining on skin sections of WT and EGFR^{ΔEgr2} skin at 5 M of age. (H) Immunofluorescence staining of SOCS3 in green of WT and EGFR^{ΔEgr2} mouse skin sections at 3 M. (I) tSNE FACS gating strategy of MHC-I and MHC-II expression among CD45⁺ keratinocytes of WT or EGFR^{ΔEgr2} at 5 M of age. (J) Real-time PCR of MHC-I expression (H-2Kb) of sorted CD34⁺ Scal⁺ HFSCs of WT or EGFR^{ΔEgr2} mice at 1 M of age ($p = 0.0229$). (K) CD55 ($p = 0.0481$) and CD200 ($p = 0.001$) surface expression in HFSCs and HF of WT and EGFR^{ΔEgr2} mice by FACS analysis. Data is presented in $\pm$ SEM, * $p < 0.05$, ** $p < 0.01$ by unpaired t-test, $n \geq 3$.





Figure EV3. Single-cell analysis identified hair follicle and immune cell populations in EGFR^{ΔEgr2} mouse epidermal cell suspensions and cytokine impact of HF specific MHC expression.

(A) Top 3 differentially expressed genes of every cluster of single-cell RNA sequencing analysis of epidermal CD45⁺ immune cells and CD45⁺ Sca-1⁺ hair follicle cells in EGFR^{ΔEgr2}. Wilcoxon rank sum test and Bonferroni correction for statistical analysis. (B) Ex vivo WT skin explants treated with indicated cytokines with or without erlotinib for 48 h. FACS analysis of MHC-I and -II expressions in CD200⁺ HF cells. (C) Top 30 differentially expressed genes of CD8 and NK cell cluster. (D) Confirmation of CD8 T cell (WT $p = 0.0481$, EGFR^{ΔEgr2} $p = 0.0246$) and (E) NK cell depletion by FACS analysis of EGFR^{ΔEgr2} mice and their respective controls. (F) IF staining of CD34⁺ and Sox9⁺ stem cells in skin sections of EGFR^{ΔEgr2} treated with the indicated depletion antibodies and the respective controls. Data is presented in $\pm$ SEM, * $p < 0.05$ by One-Way ANOVA with Tukey's posthoc correction, $n \geq 3$.

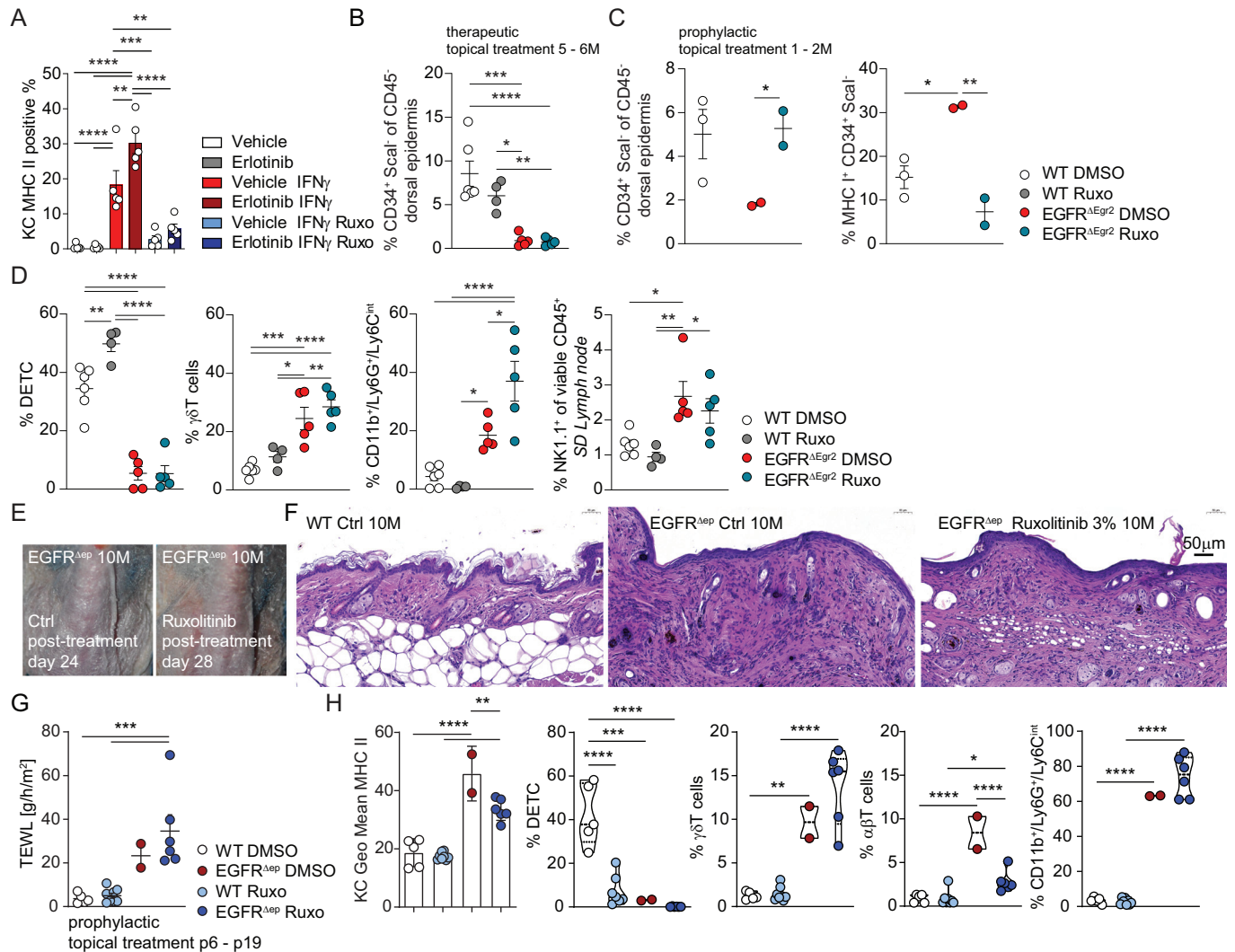


Figure EV4. Therapeutic and prophylactic JAK inhibition ameliorates skin inflammation in EGFR Δ Egr2 and EGFR Δ ep mice.

(A) MHC-II expression of in vitro primary murine KCs of WT mice treated with IFN γ and JAK1/2 inhibitor (ruxolitinib) with or without the EGFR-inhibitor erlotinib (Veh vs Veh IFN γ , Veh vs Erlotinib IFN γ , Erlotinib vs Veh IFN γ and Erlotinib vs Erlotinib IFN γ $p < 0.0001$, Veh IFN γ vs Erlotinib IFN γ $p = 0.0085$, Veh IFN γ vs Erlotinib IFN γ Ruxo $p = 0.0005$, Veh IFN γ vs Erlotinib IFN γ Ruxo $p = 0.0054$, Erlotinib IFN γ vs Veh IFN γ Ruxo and Erlotinib IFN γ vs Erlotinib IFN γ Ruxo $p < 0.0001$). (B) Summary of FACS analysis of CD34 $^{+}$ hair follicle stem cells from 5 M old WT and EGFR Δ Egr2 mice treated therapeutically with DMSO or ruxolitinib in DMSO for 1 month (WT vs EGFR Δ Egr2 $p = 0.0001$, WT vs EGFR Δ Egr2 Ruxo $p < 0.0001$, EGFR Δ Egr2 vs WT Ruxo $p = 0.0121$, WT Ruxo vs EGFR Δ Egr2 Ruxo $p = 0.0098$). (C) FACS analysis of HFSC ($p = 0.0493$) and MHC-I expression (WT vs EGFR Δ Egr2 $p = 0.0224$, EGFR Δ Egr2 vs EGFR Δ Egr2 Ruxo $p = 0.0076$) on EGFR Δ Egr2 and WT mice treated prophylactically with 3% ruxolitinib from 1 to 2 M of age. (D) FACS analysis of epidermal CD45 $^{+}$ immune cells of EGFR Δ Egr2 and WT treated therapeutically with 3% Ruxolitinib from 5 M to 6 M (DETC: WT vs WT Ruxo $p = 0.0088$, other $p < 0.0001$, $\gamma\delta$ T cells: WT vs EGFR Δ Egr2 $p = 0.0004$, WT vs EGFR Δ Egr2 Ruxo $p < 0.0001$, EGFR Δ Egr2 vs WT Ruxo $p = 0.0115$, WT Ruxo vs EGFR Δ Egr2 $p = 0.0012$, CD11b/Ly6G $^{+}$ /Ly6C int : WT vs EGFR Δ Egr2 Ruxo and WT Ruxo vs EGFR Δ Egr2 Ruxo $p < 0.0001$, EGFR Δ Egr2 vs WT Ruxo $p = 0.0265$, EGFR Δ Egr2 vs EGFR Δ Egr2 Ruxo $p = 0.0131$, WT vs EGFR Δ Egr2 $p = 0.0142$, EGFR Δ Egr2 vs WT Ruxo $p = 0.0056$, WT Ruxo vs EGFR Δ Egr2 Ruxo $p = 0.0381$). (E, F) Representative pictures of the skin (E) and H&E stainings (F) of WT, EGFR Δ Egr2 mice treated topically with DMSO (vehicle ctrl) or 3% ruxolitinib in DMSO daily at the age of 10 M for 4 weeks. (G, H) WT and EGFR Δ ep mice were treated prophylactically with 3% Ruxolitinib or DMSO starting from P6 until P19 and TEWL (WT vs EGFR Δ Egr2 Ruxo $p = 0.0007$, WT Ruxo vs EGFR Δ Egr2 Ruxo $p = 0.0003$) was measured from the back-skin. (H) FACS analysis of these mice for inflammatory parameters (KC MHC II: EGFR Δ Egr2 vs EGFR Δ Egr2 Ruxo $p = 0.0056$, other p values < 0.0001 , DETC: all p values < 0.0001 , $\gamma\delta$ T cells: WT vs EGFR Δ Egr2 $p = 0.0036$, EGFR Δ Egr2 vs WT Ruxo $p = 0.0027$, other $p < 0.0001$, $\alpha\beta$ T cells: WT vs EGFR Δ Egr2 Ruxo $p = 0.0429$, WT Ruxo vs EGFR Δ Egr2 Ruxo $p = 0.0191$, other $p < 0.0001$, CD11b/Ly6G $^{+}$ /Ly6C int : all p values < 0.0001) as indicated. Data is presented in $\pm$ SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ by one-way ANOVA with Tukey's posthoc correction, $n \geq 3$.

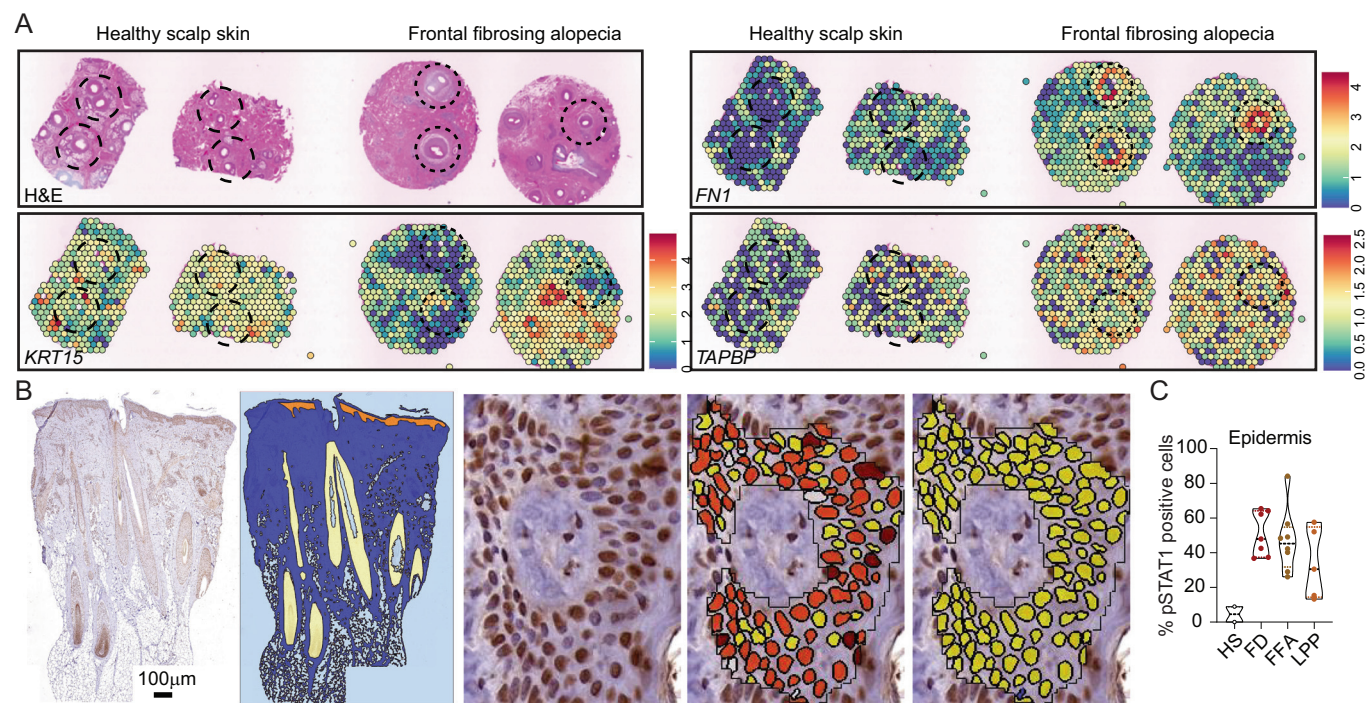


Figure EV5. Key features of scarring alopecia in spatial transcriptomics and quantification of phosphorylated STAT1 in human clinical samples.

(A) Spatial transcriptomic feature plots of the indicated genes superimposed on the corresponding H&E images. The H&E image is also available at Fig. 6C. Dataset from Cohen et al (Data ref: Cohen et al, 2024). (B) Nuclear staining intensity was quantified using definens software as indicated. Hair follicles (yellow area) and epidermis (orange area) were separately analyzed. Medium intensity is shown in red. (C) Quantification of pSTAT1 staining intensity in the epidermis, $n \geq 2$.