Science
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Supplementary Materials for

The use of ectopic volar fibroblasts to modify skin identity

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The PDF file includes:

Figs. S1 to S27
Tables S2, S4, S18, and S19

Other Supplementary Material for this manuscript includes the following:

Tables S1, S3, S5 to S17, S20, and S21
Movies S1 to S5
MDAR Reproducibility Checklist

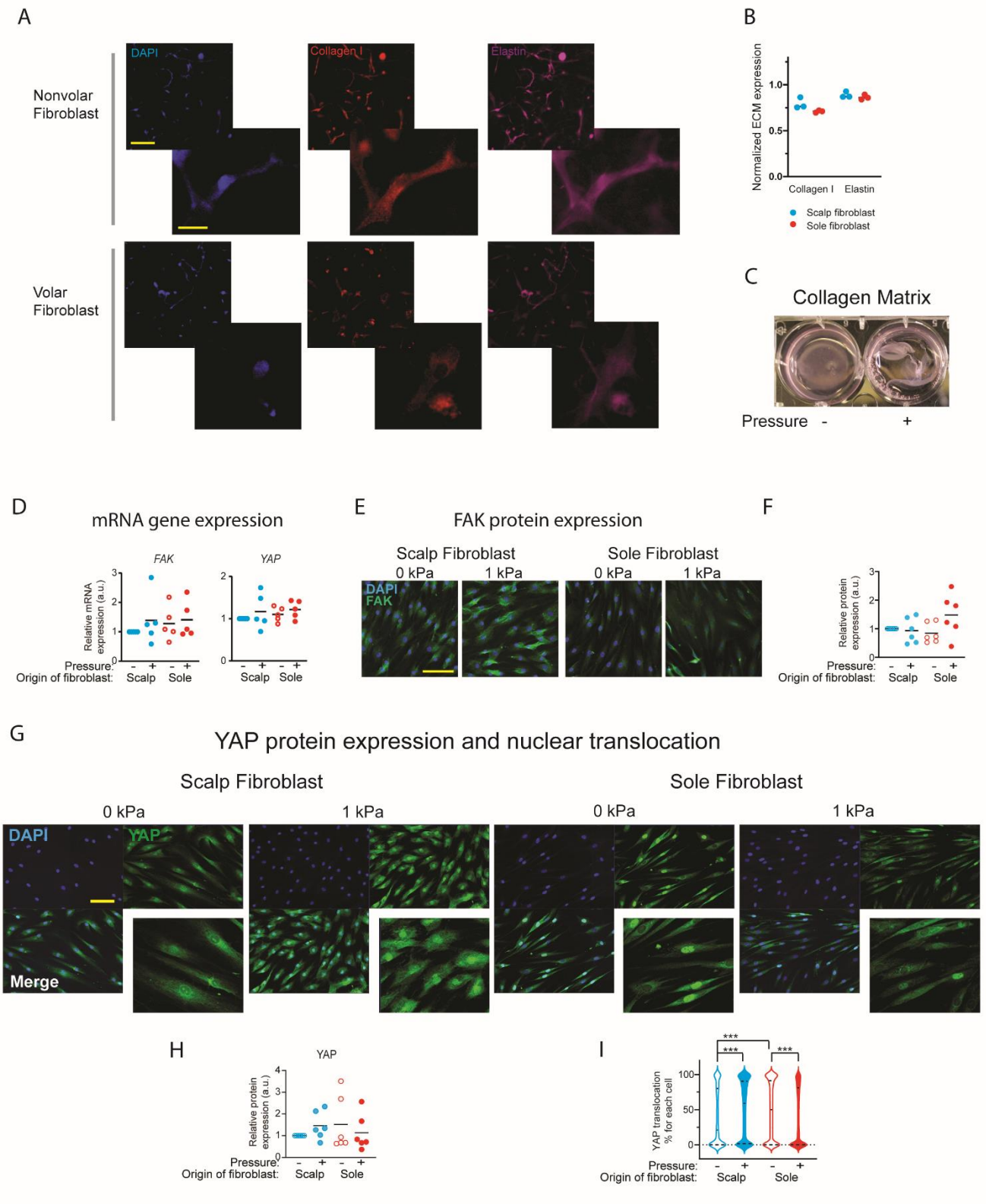

A
Nonvolar Fibroblast
Volar Fibroblast
DAPI
Collagen I
Elastin
B
Normalized ECM expression
1.0
0.5
0.0
Collagen I
Elastin
Scalp fibroblast
Sole fibroblast
C
Collagen Matrix
Pressure - +
D
mRNA gene expression
FAK
YAP
Relative mRNA expression (a.u.)
Pressure: - + - +
Origin of fibroblast: Scalp Sole
E
FAK protein expression
Scalp Fibroblast
Sole Fibroblast
0 kPa
1 kPa
DAPI
FAK
F
Relative protein expression (a.u.)
Pressure: - + - +
Origin of fibroblast: Scalp Sole
G
YAP protein expression and nuclear translocation
Scalp Fibroblast
Sole Fibroblast
0 kPa
1 kPa
DAPI
YAP
Merge
H
YAP
Relative protein expression (a.u.)
Pressure: - + - +
Origin of fibroblast: Scalp Sole
I
***
***
***
YAP translocation % for each cell
Pressure: - + - +
Origin of fibroblast: Scalp Sole

Fig. S1. Fibroblast 3D cultures in collagen gel and Expression of FAK and YAP in scalp and sole fibroblast in response to dynamic pressure. (A) Representative images of non-volar (scalp) or volar (sole) fibroblast 3D cultures in collagen gel (n = 3 unique subjects) stained for collagen I and elastin. Scale bar = 10 μm. (B) The scatter plot shows the expressions of collagen I and elastin in scalp and sole fibroblast (p = n.s.). (C) 3-D collagen matrices before and after pressure treatment. (D) Scatter plots show *FAK* and *YAP* mRNA expression in scalp or sole fibroblast in response to dynamic pressure (p = n.s.). n = 5 unique subjects with two repeats. (E) Representative images of protein FAK expression in scalp and sole fibroblasts in response to dynamic pressure. Scale bar = 50 μm. (F) The scatter plot shows FAK protein expression in scalp or sole fibroblast in response to dynamic pressure (p = n.s.). n = 6 unique subjects. (G) Representative images of protein YAP expression and YAP protein into nucleus translocation in scalp or sole fibroblast in response to dynamic pressure. Scale bar = 50 μm. (H) The scatter plot shows YAP protein expression in scalp or sole fibroblast in response to dynamic pressure (p = n.s.). n = 6 unique subjects. (I) The scatter plot shows YAP nuclear translocation was significantly higher in sole fibroblast compared to scalp fibroblast ($p < 0.001$). In response to dynamic pressure, YAP nuclear translocation was significantly increased in non-volar fibroblasts ($p < 0.001$) and significantly decreased in volar fibroblasts ($p < 0.001$). n = 5000 cells from 6 unique subjects.

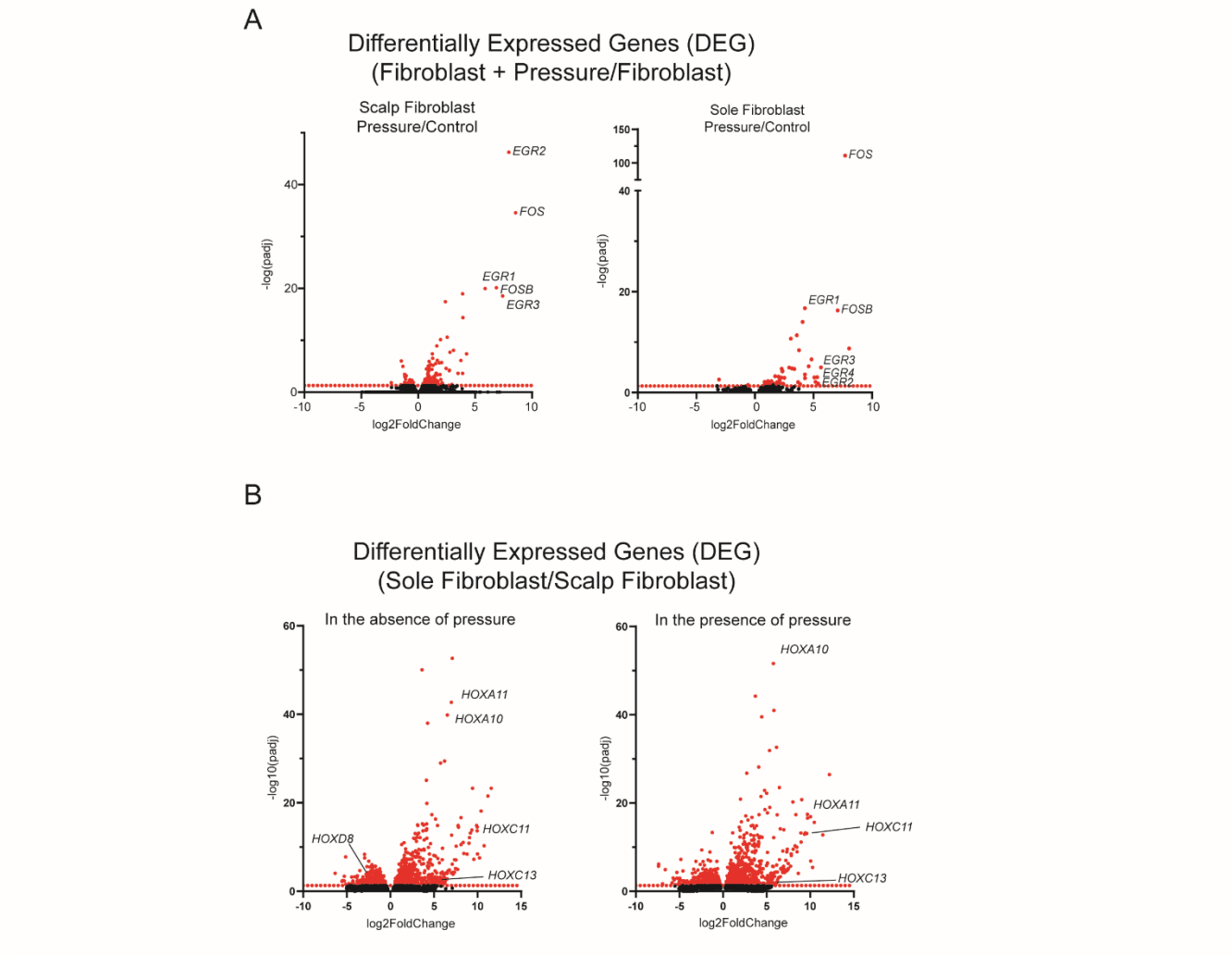


Fig S2. Differentially expressed genes (DEG) of scalp and sole fibroblasts in response to dynamic pressure using bulk RNA seq. (A) The volcano plots of DEG in scalp or sole fibroblasts under dynamic pressure. Significantly up- or down-regulated genes ($p < 0.05$) are highlighted in red. Genes significantly upregulated by pressure treatment in both scalp and sole fibroblasts are labeled. (B) The volcano plots of DEG in sole fibroblast compared to scalp fibroblast in the presence or absence of pressure. Significantly up- or down-regulated genes ($p < 0.05$) are highlighted in red. *HOX* genes significantly up- or down-regulated by pressure treatment are labeled.

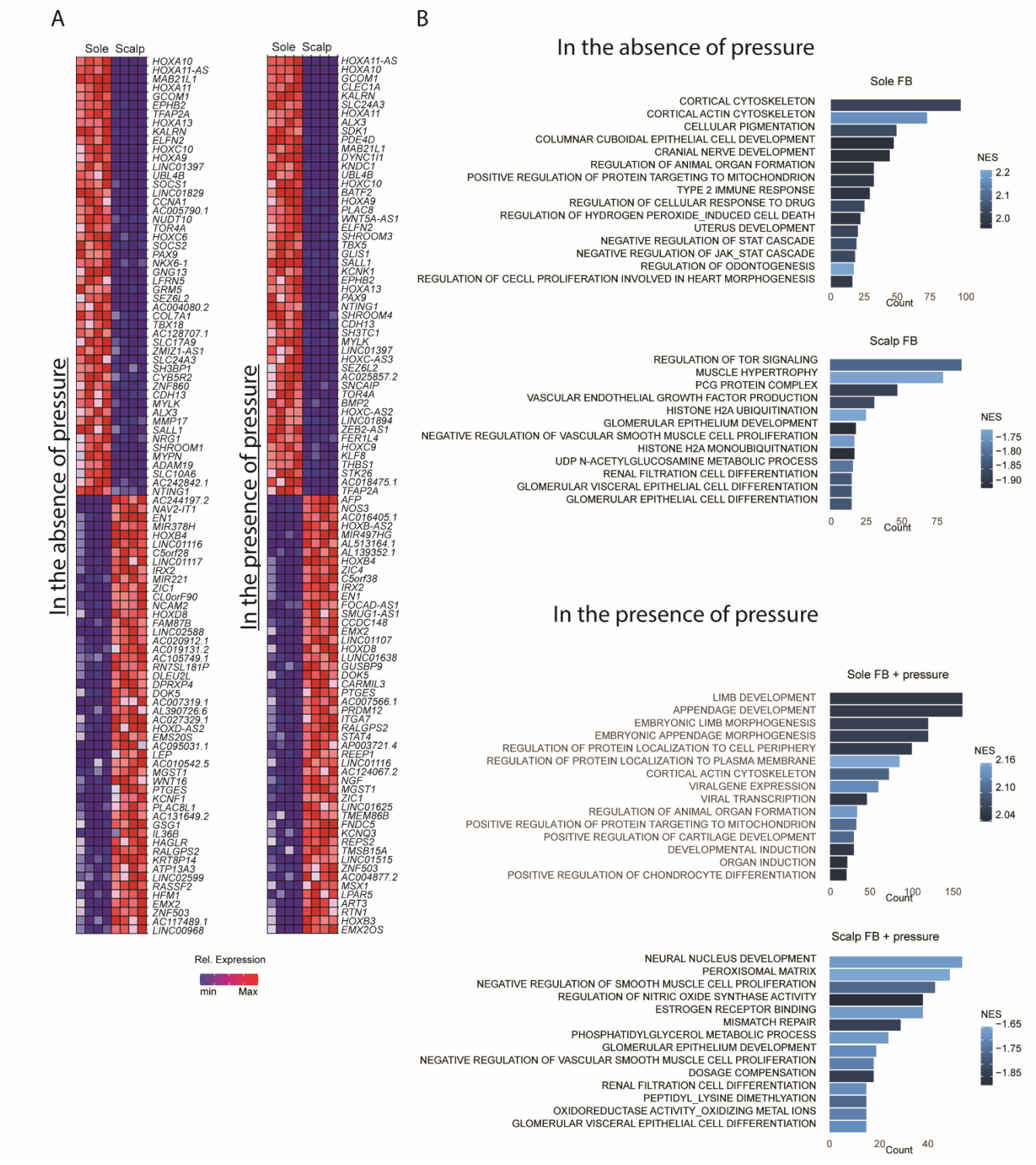


Fig. S3. Gene Set Enrichment Analysis (GSEA) of scalp and sole fibroblast in response to dynamic pressure. (A) Heatmaps illustrating modification of gene expression in scalp and sole fibroblasts at baseline and in response to dynamic pressure. Blue cells indicate a minimum relative expression level in the target while red cells indicate a maximum. (B) Gene Ontology (GO) pathways for each condition. Normalized enrichment scores (NES) for scalp

GO pathways illustrate weaker expression in both presence and absence of pressure. The addition of pressure to sole fibroblast samples results in an increase in a higher count and enrichment of limb developmental pathways.

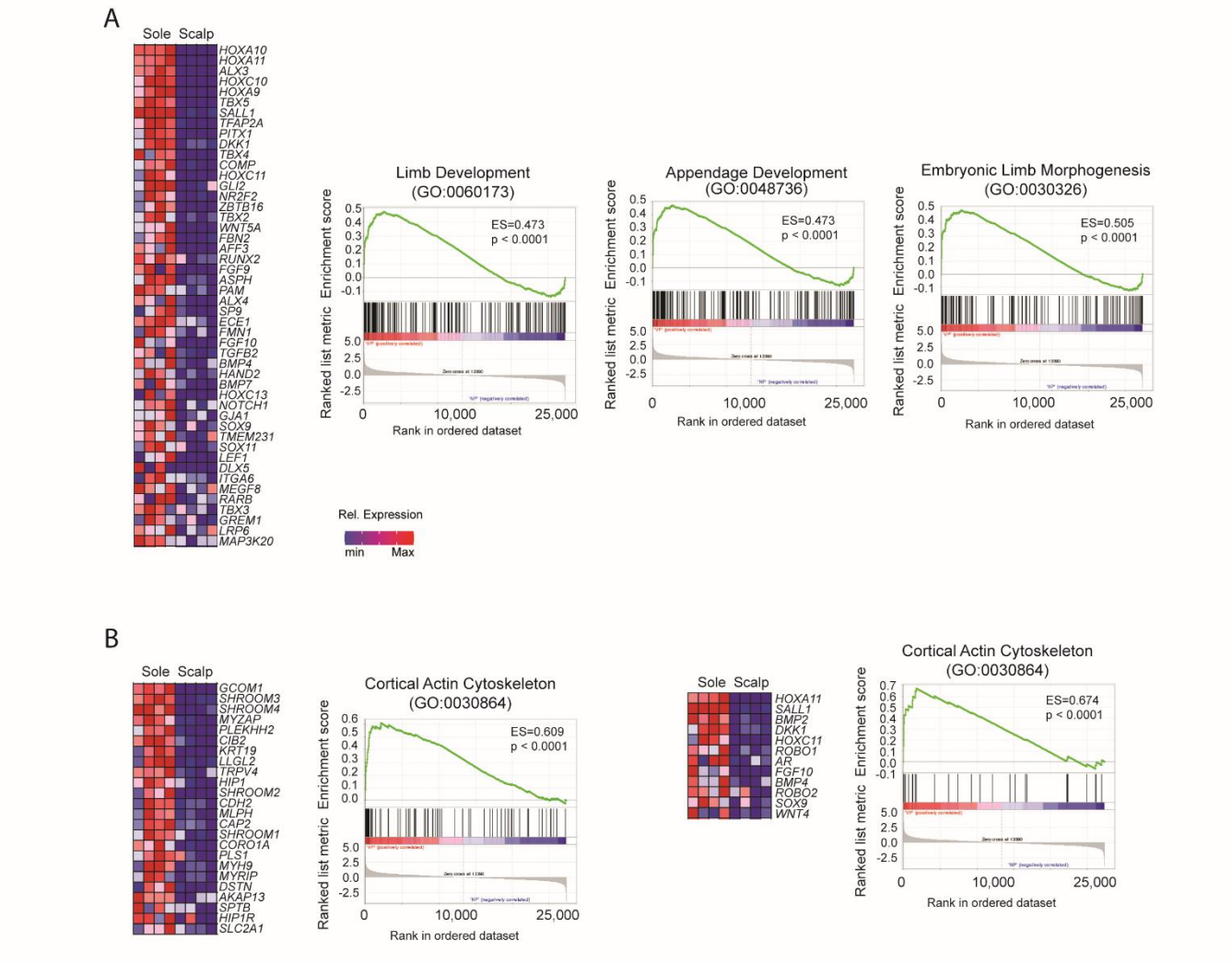


Fig. S4. Enrichment plot of selected top GSEA pathway from fig. S3. (A) Heatmap showing modification of gene expression associated with GO pathways of limb development, appendage development and embryonic limb morphogenesis in scalp and sole fibroblasts in response to dynamic pressure. (B) Heatmap showing modification of gene expression associated with GO pathways of cortical actin cytoskeleton and cortical cytoskeleton in scalp and sole fibroblasts in response to dynamic pressure.

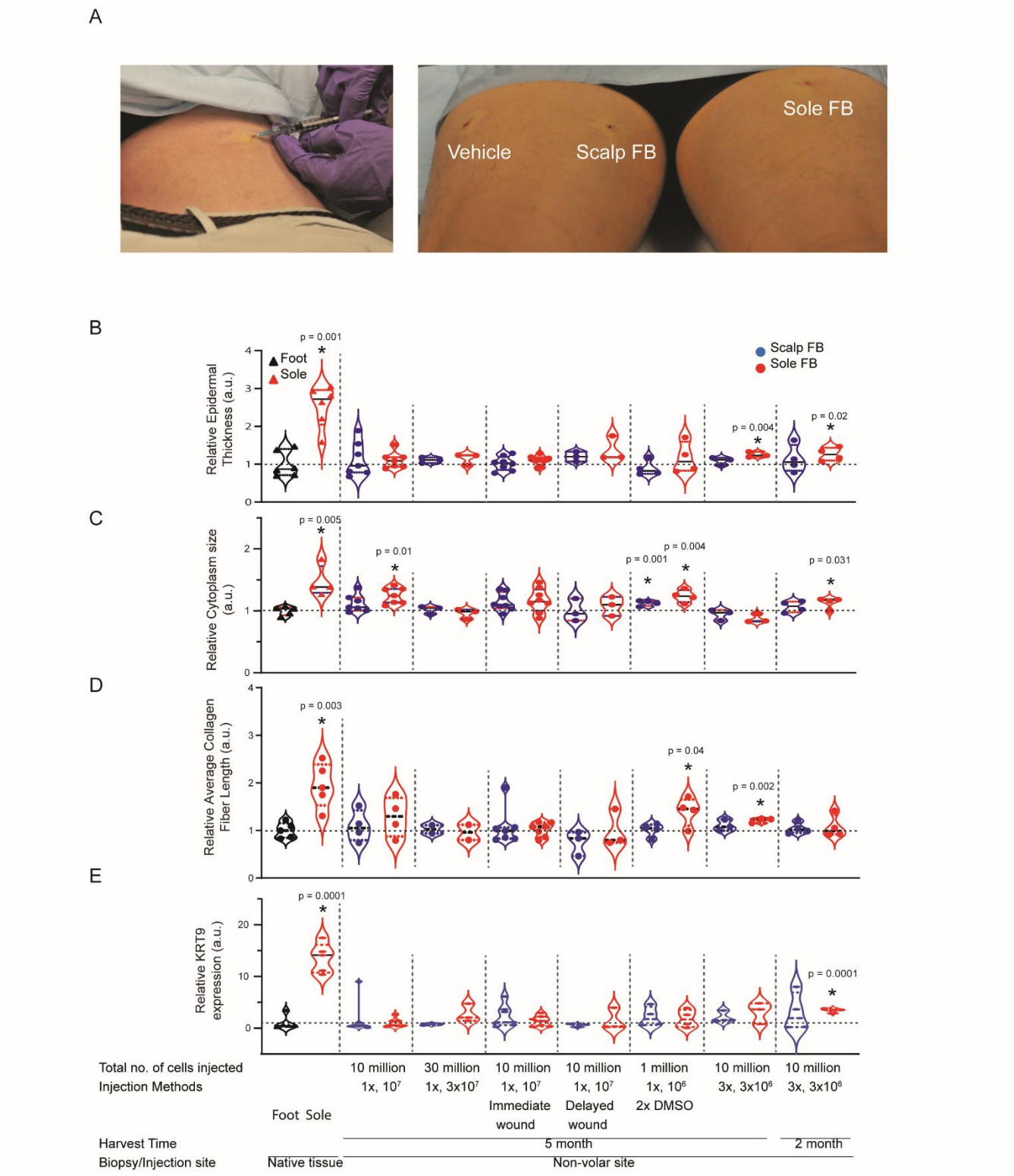


Fig S5. Image of vehicle or cell injection and violin plot distributions illustrating variance in post-injection. (A) Images of vehicle and cell injections on test site. (B) Violin plot showing relative epidermal thickness in scalp and sole fibroblast injection site compared to vehicle injection site. Significant increases in relative epidermal

thickness occur at 5 months with three fractionated sole FB injections dosed at $3.3x10^6$ cells ($p = 0.004$, $n = 3$ unique subjects) for a total of 10 million cells. This increase in thickness can be noted as early as 2 months post-injection ($p = 0.02$, $n = 4$ unique subjects). (C) Violin plot showing relative cytoplasm size of keratinocytes in scalp and sole fibroblast injection site compared to vehicle injection site. Significant changes in cytoplasm size can be observed with doses as low as 1 million cells with the addition of two DMSO injections in both scalp FB and sole FB injection sites ($p = 0.001$, $p = 0.004$, $n = 4$ unique subjects). Notably, the sole FB injection site results in a higher median size, and this increase can also be noted at 2 months post-injection with 10 million cells fractionated over three injections ($p = 0.031$, $n = 4$ unique subjects) as well as after 5 months with a single 10 million cell dose ($p = 0.01$, $n = 7$ unique subjects). (D) Violin plot showing relative average dermal collagen length in scalp and sole fibroblast injection site compared to vehicle injection site. Significant increases in the relative average collagen fiber length occur with both a single sole FB injection dosed at 1 million cells with two injections of DMSO as well as 10 million sole FB fractionated over three injections ($p = 0.04$, $n = 4$ unique subjects; $p = 0.002$, $n = 3$ unique subjects). (E) Violin plot showing relative KRT9 protein expression in scalp and sole fibroblast injection site compared to vehicle injection site. Relative KRT9 expression increases significantly with fractionated sole FB administered in three doses of $3.3x10^6$ cells for a total of 10 million cells at 2 months ($p = 0.0001$, $n = 4$ unique subjects).

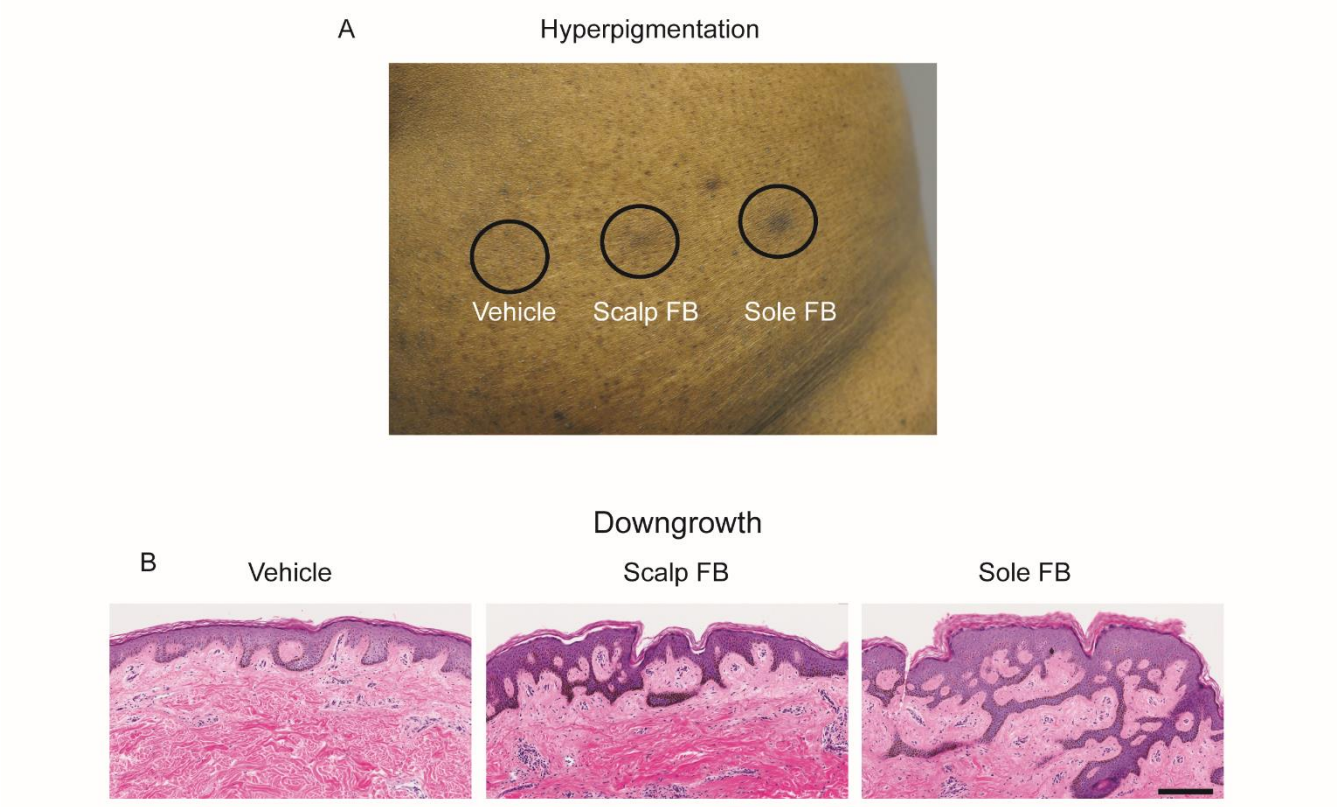


Fig S6. Side effects of high dose (30 million cells in single injection). (A) Image showing hyperpigmentation due to high in sole fibroblast injection site (Sole FB). (B) H&E images of abnormal downgrowth of epidermis with high dose of scalp or sole fibroblast injections. Scale bar = 100 μm.

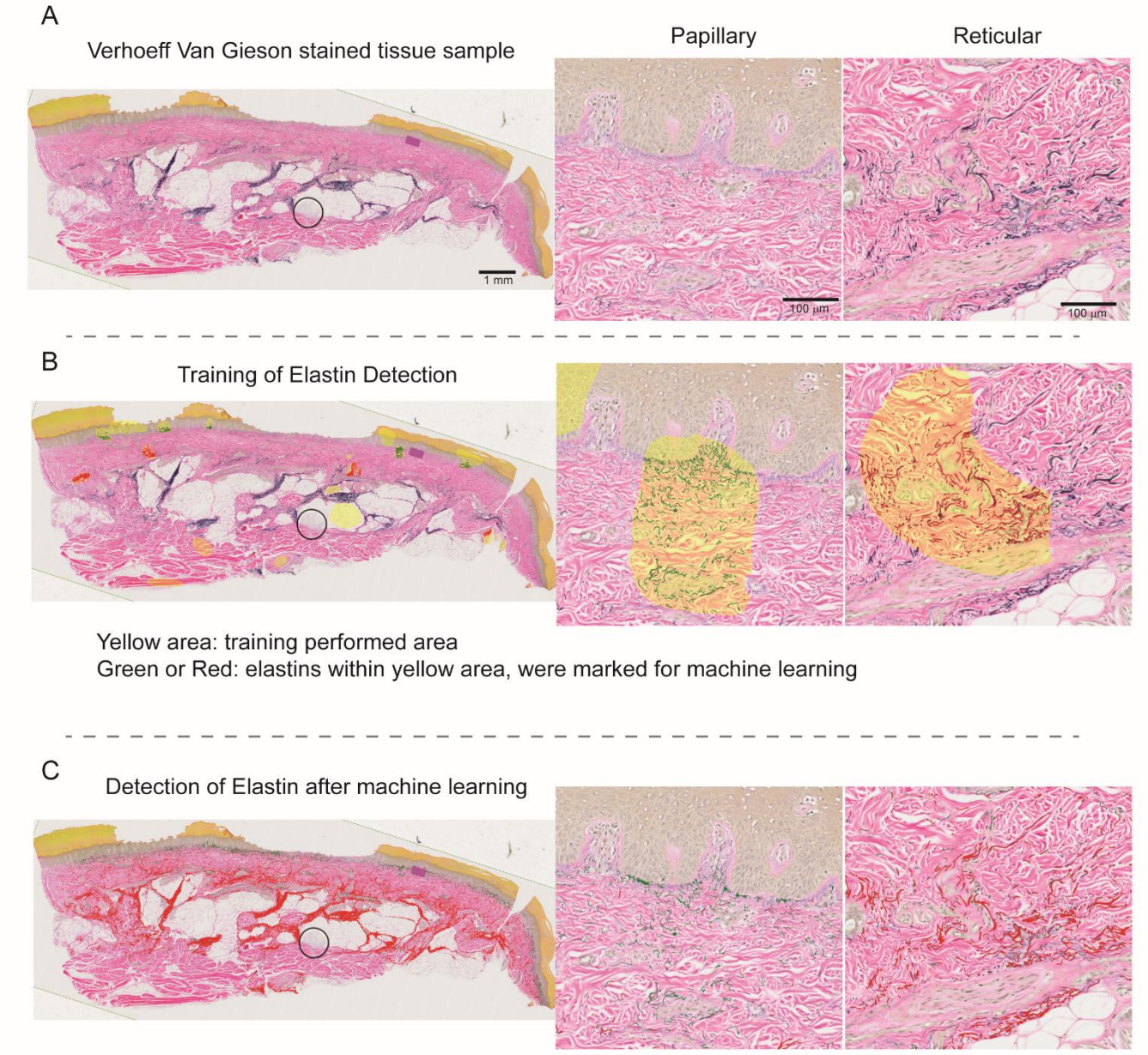


Fig S7. Detection of elastin by AI machine learning. (A) Verhoeff Van Gieson stained elastin (dark purple) on the tissue sample. Entire tissue image (left) and zoomed papillary and reticular regions (right). (B) The yellow-circled regions were chosen for training. Within the yellow-circled region, thin elastin fibers in the papillary regions (total of 7 areas) were highlighted in green and thick elastin fibers in the reticular regions (total of 11 areas) were highlighted in red for machine training. Elastin-free regions (within the epidermis or fat layer) were included for training purposes. (C) Detection of elastin after machine training. Elastins were detected based on color and thickness. The AI highlighted papillary elastins in green and reticular elastins in both green and red. Further analysis of papillary elastins were chosen based on their location.

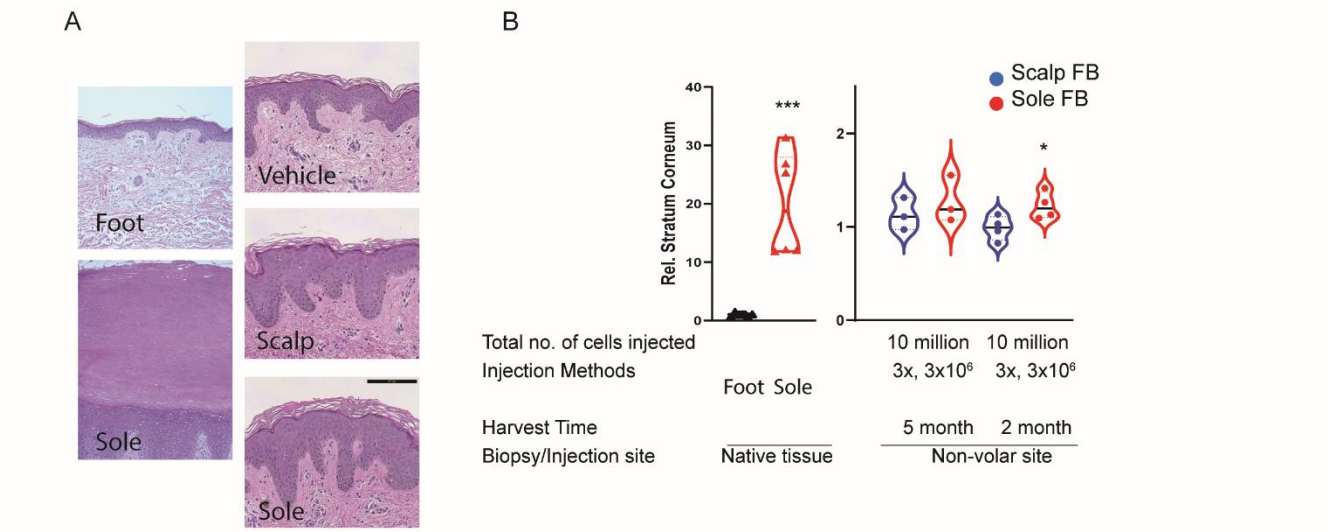


Fig S8. Stratified corneum changes in human trial. (A) Representative images of H&E stained tissues from native and injection sites. Scale bar = 100 µm. (B) Relative stratified corneum layer thickness in native volar (sole) skin was significantly greater compared to non-volar (foot) skin ($p < 0.0001$, n = 6 unique subjects with one repeat). There was significant difference at 2-month post-injection of fractionated sole fibroblast compared to Vehicle site ($p = 0.04$, n = 4 unique subjects).

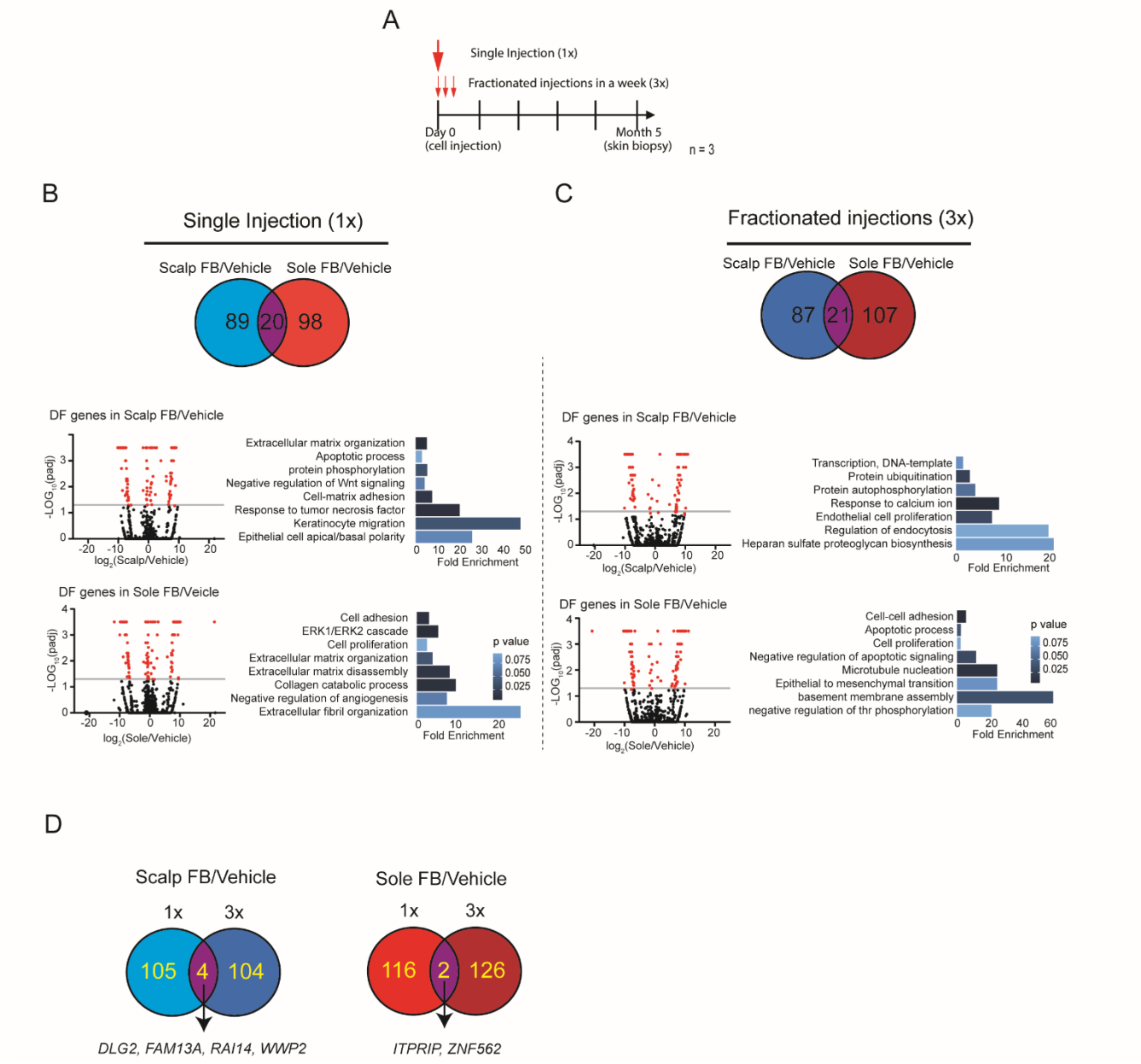


Fig S9. Comparison of single and fractionated injection methods by differential gene analysis. (A) Timeline of injection administration using single or fractionated (3x) method. Venn diagram of (B) DEG in single injection groups and (C) fractionated injection groups with volcano plots and GO pathways. (D) Venn diagrams illustrate the commonly modified genes between DEGs in Scalp FB/Vehicle from single and multiple injections, or in Sole FB/Vehicle. The commonly modified genes are *DLG2* (upregulated), *RAI14* (upregulated), *WWP2* (upregulated), and *FAM13A* (up in 1x and down in 3x) in Scalp FB/Vehicle group and *ITPRIP* (upregulated) and *ZNF562* (downregulated) in Sole FB/Vehicle group.

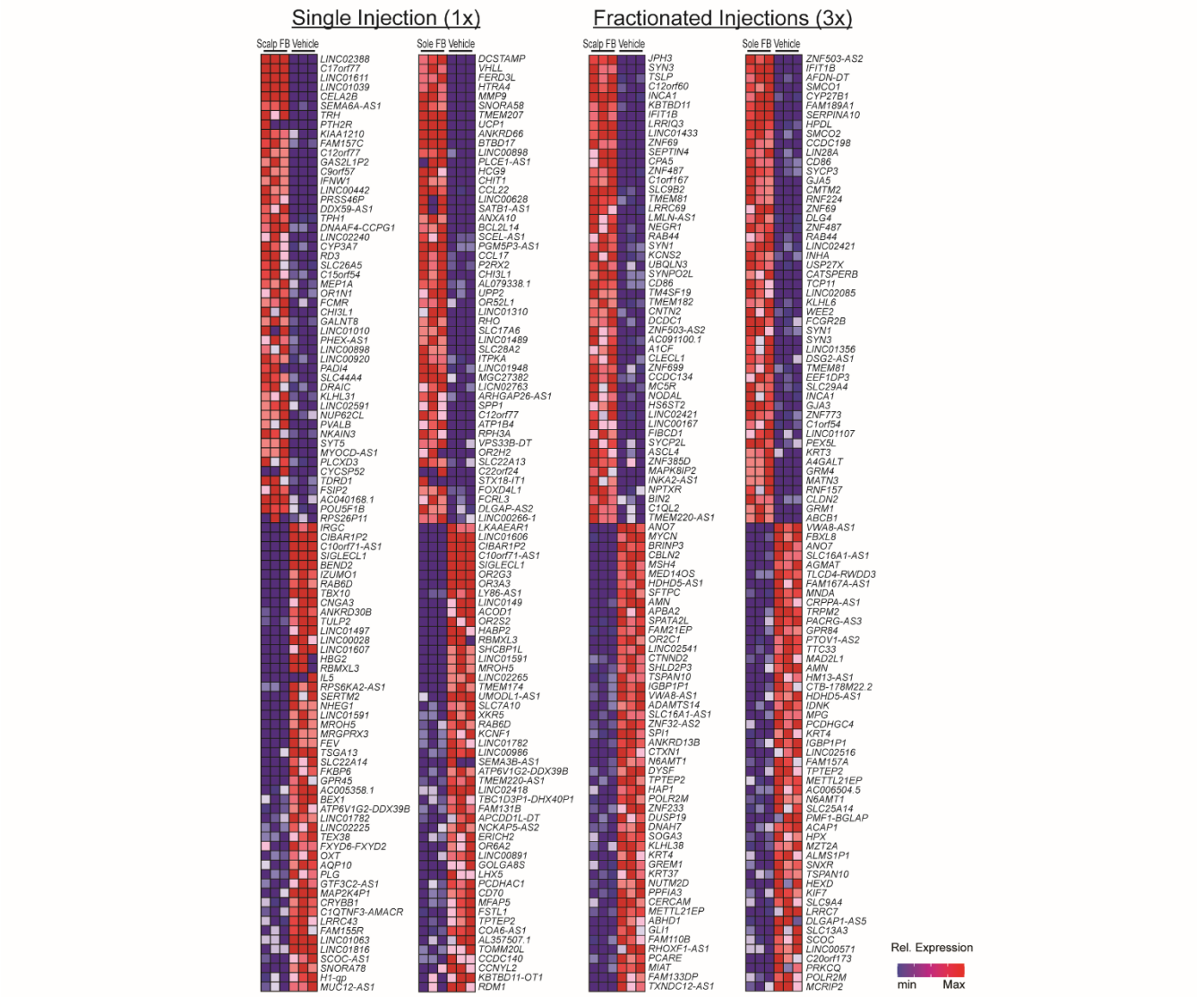


Fig. S10. Comparison of single and fractionated injection methods: Heatmap of top 100 genes by GSEA. Comparison of relative gene expression in single or fraction injections of Scalp FB/Vehicle and Sole FB/Vehicle.

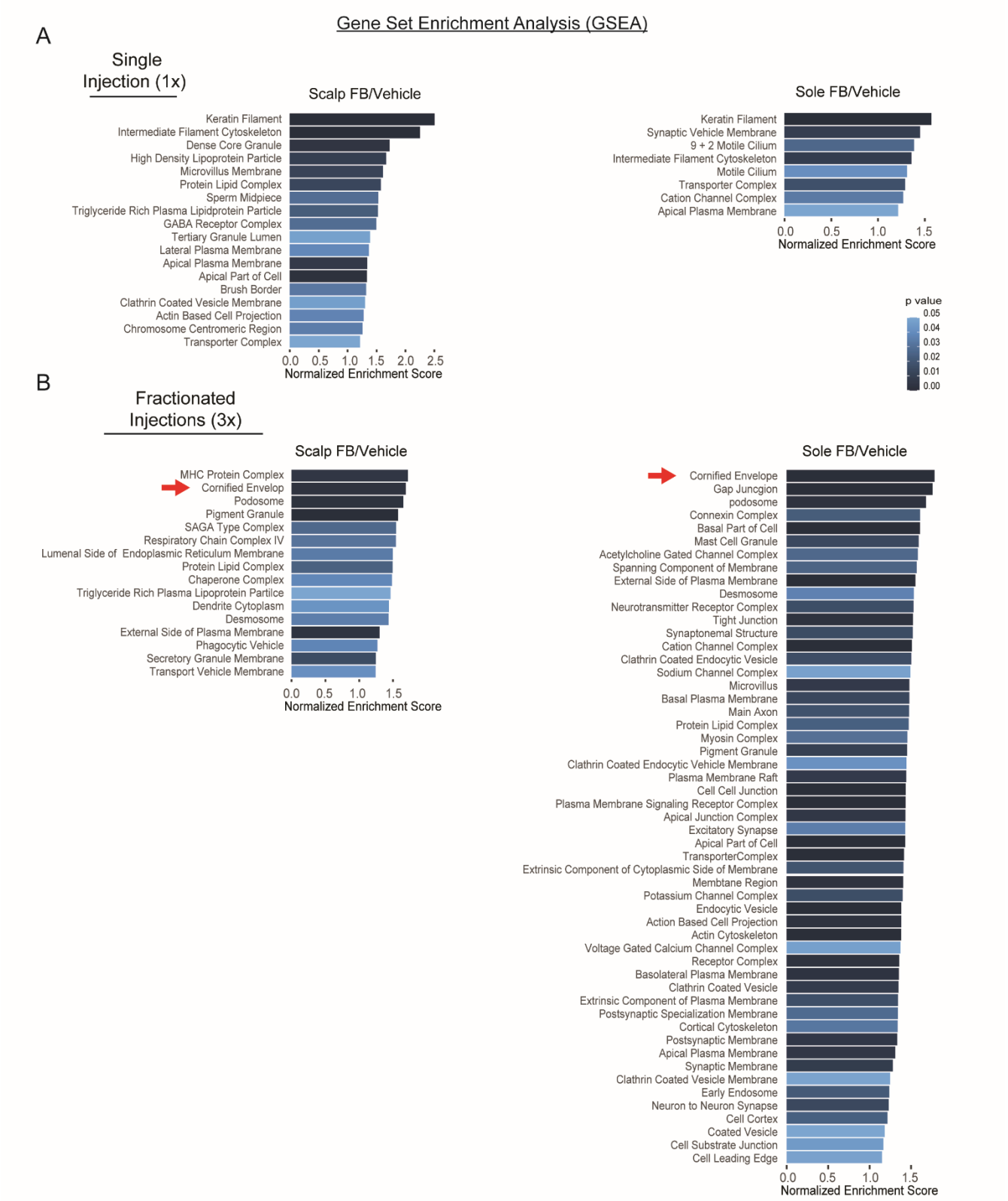


Fig. S11. Comparison of single and fractionated injection methods: GO pathways by GSEA. (A) GO pathways identified by GSEA for Scalp FB/Vehicle and Sole FB/Vehicle administered through single or (B) fractionated injections.

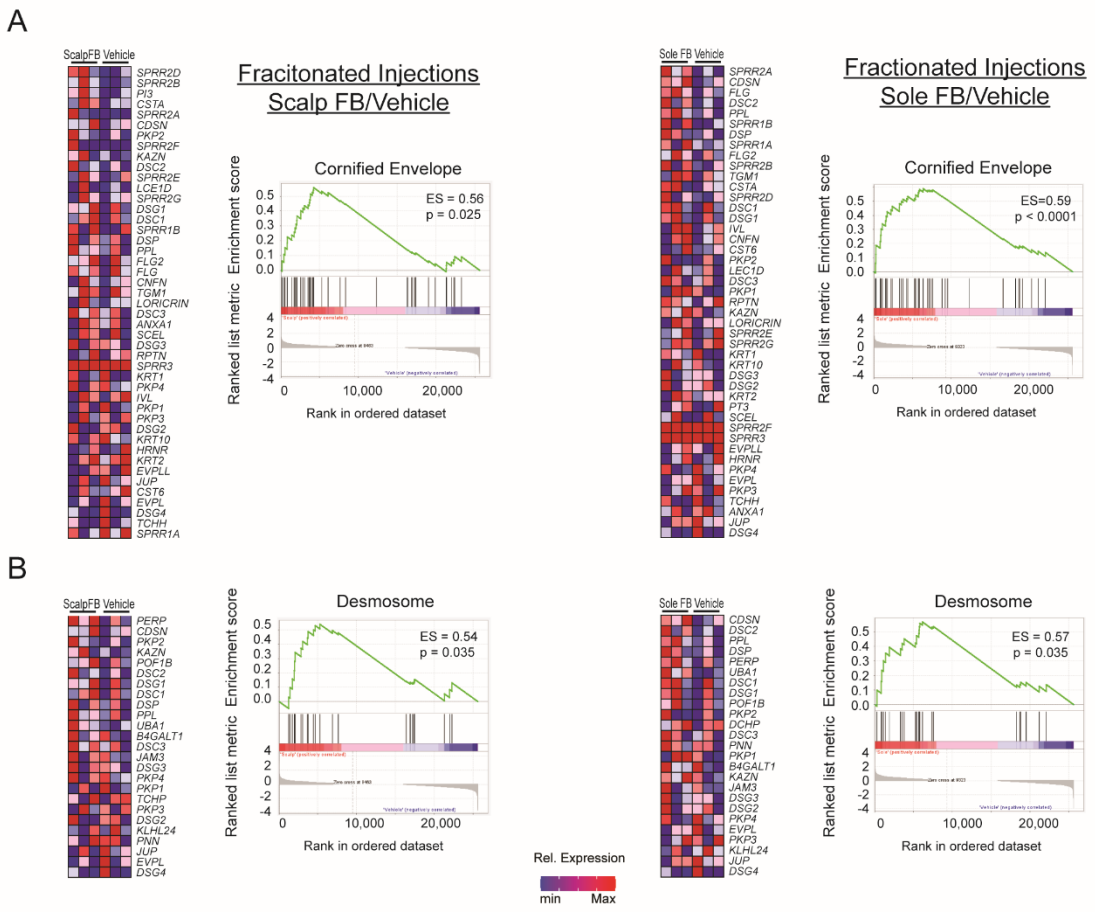


Fig. S12. Enrichment plot of selected GSEA pathway in fig. S11. (A) Cornified envelope gene set enrichment plots in fractionated injections of Scalp FB/Vehicle (ES = 0.56, p = 0.025) and Sole FB/Vehicle (ES = 0.59, p = 0.0001) (B) Desmosome gene set enrichment plots in fractionated injections of Scalp FB/Vehicle (ES = 0.54, p = 0.035) and Sole FB/Vehicle (ES = 0.57, p = 0.035).

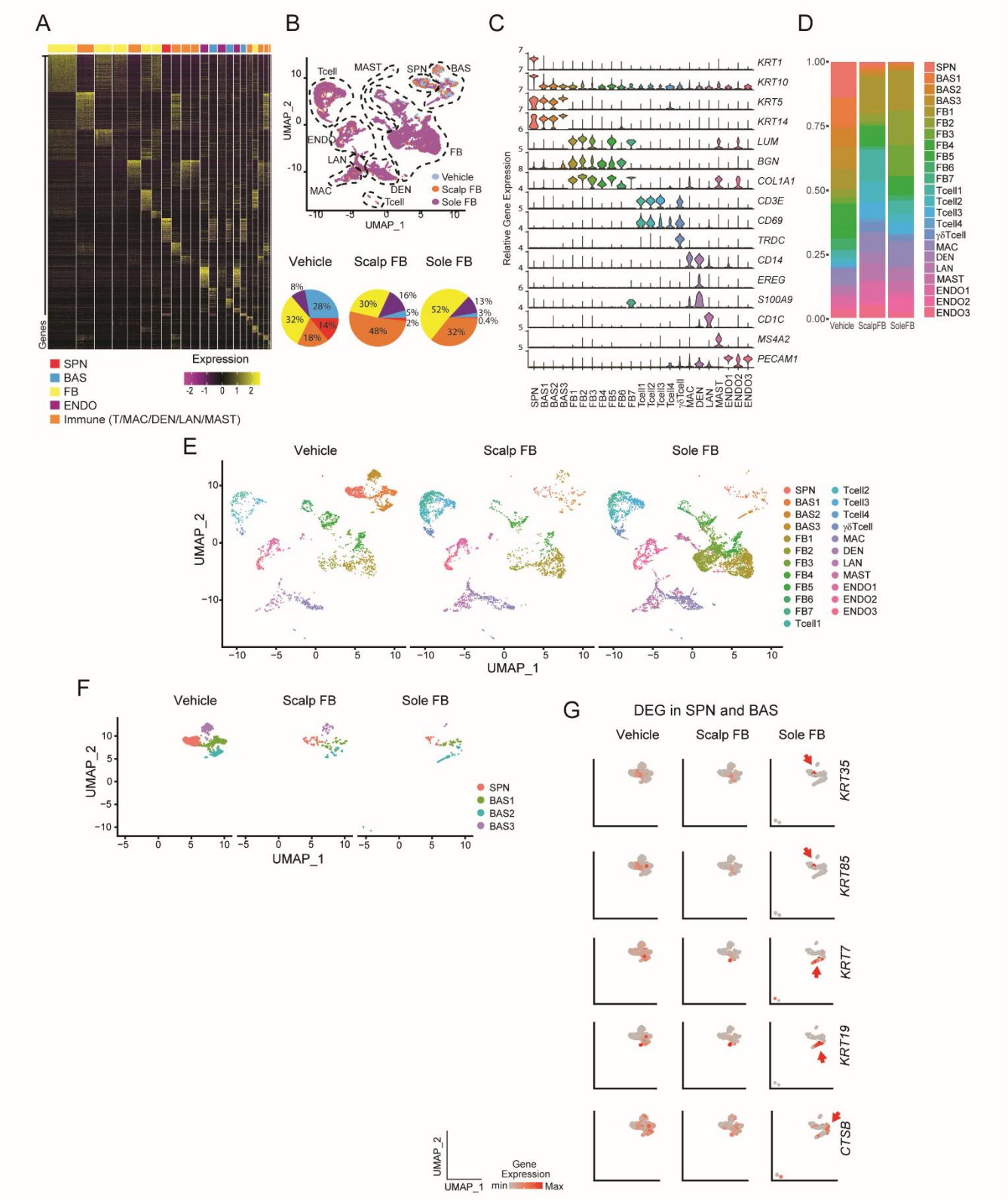


Fig. S13. Bioinformatics with scRNAseq from the 2-week post-injection time point. (A) Heat map of expression in the top 200 genes for each integrated human skin cell cluster. (B) UMAP plot shows the cluster groupings based

on gene expression. Pie charts illustrate the difference in clusters among vehicle, scalp FB, and sole FB injection sites. (C) Violin plots illustrate relative gene expression of each cluster classified using cell-type specific markers. (D) Frequency diagram of cell clusters within Vehicle, Scalp FB, and Sole FB injection sites. (E) UMAP plots of vehicle, scalp FB, and sole FB injection sites. (F) UMAP plot of keratinocytes in vehicle, scalp FB, and sole FB injection sites. (G) Dot plots of DEG show gene expression among keratinocytes in injection sites.

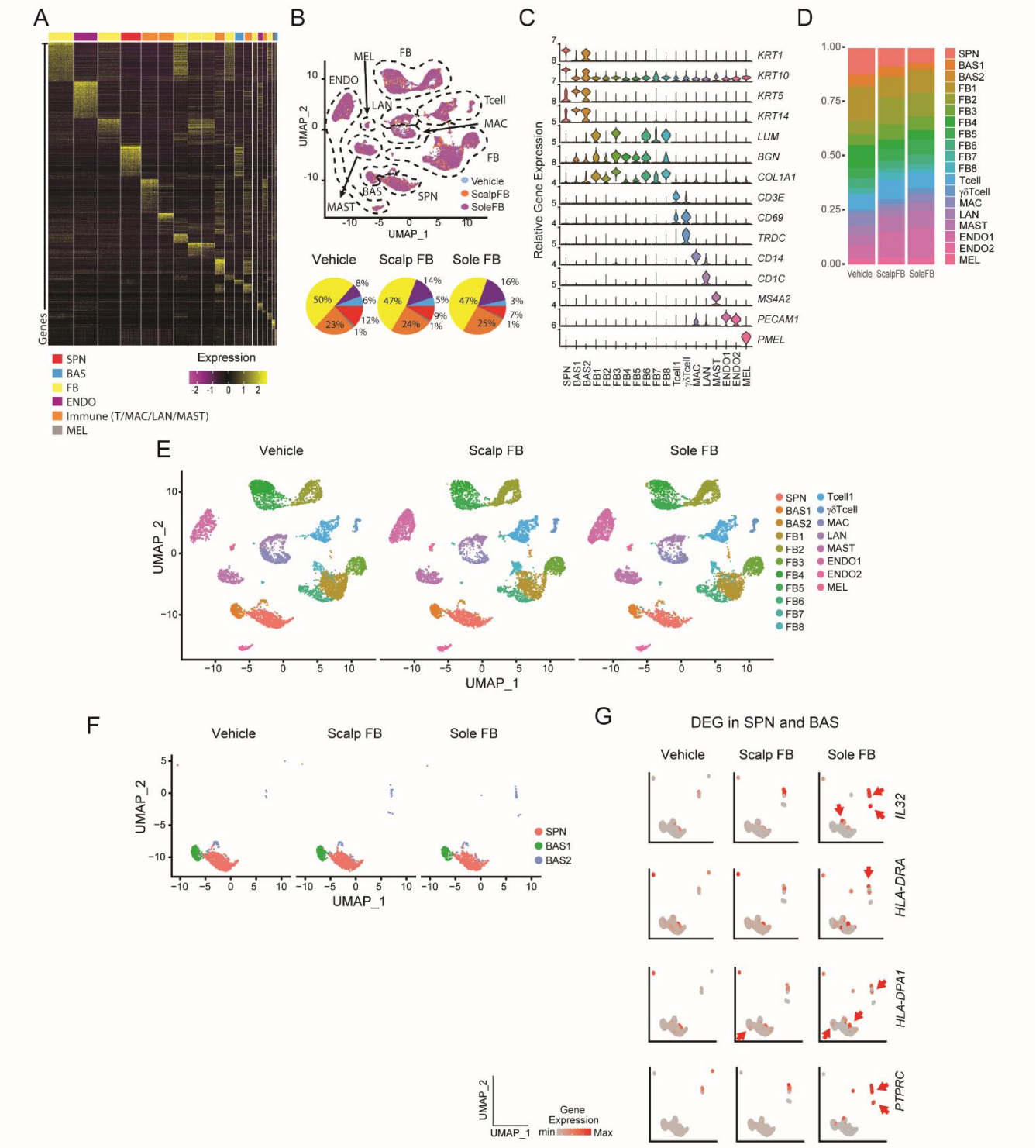


Fig. S14. Bioinformatics with scRNAseq from the 2-month post-injection time point. (A) Heat map of top 200 gene expressions. (B) UMAP of clusters and pie charts of each cluster. (C) Violin plots of marker gene expression in each cluster. (D) Frequency diagram of cell clusters. (E) UMAP plots of injection sites. (F) UMAP plot of keratinocytes. (G) Dot plot of DEG in keratinocytes.

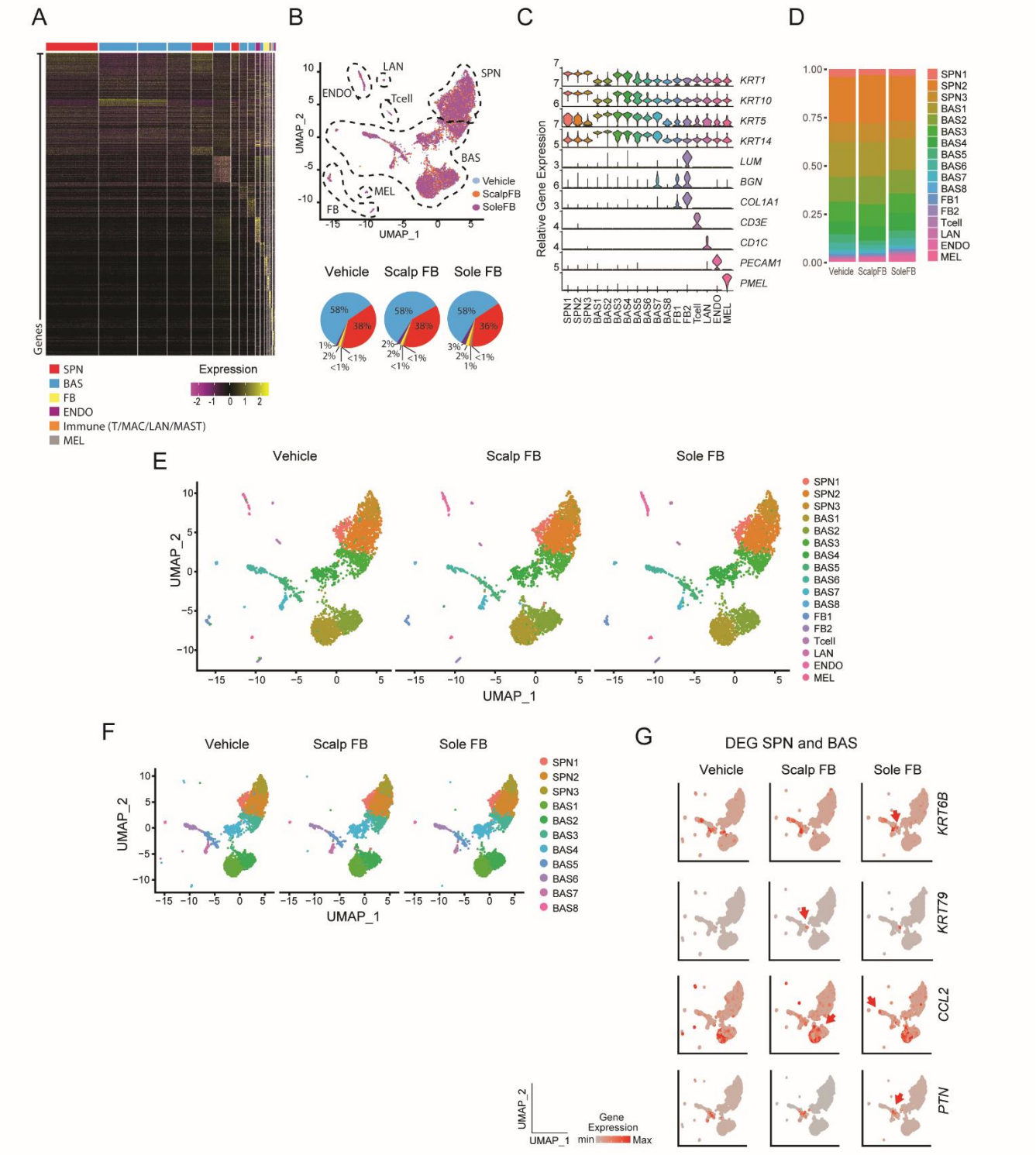


Fig. S15. Bioinformatics with scRNAseq from the 5-month post-injection time point. (A) Heat map of top 200 gene expressions. (B) UMAP of clusters and pie charts of each cluster. (C) Violin plots of marker gene expression in each cluster. (D) Frequency diagram of cell clusters. (E) UMAP plots of injection sites. (F) UMAP plot of keratinocytes. (G) Dot plot of DEG in keratinocytes.

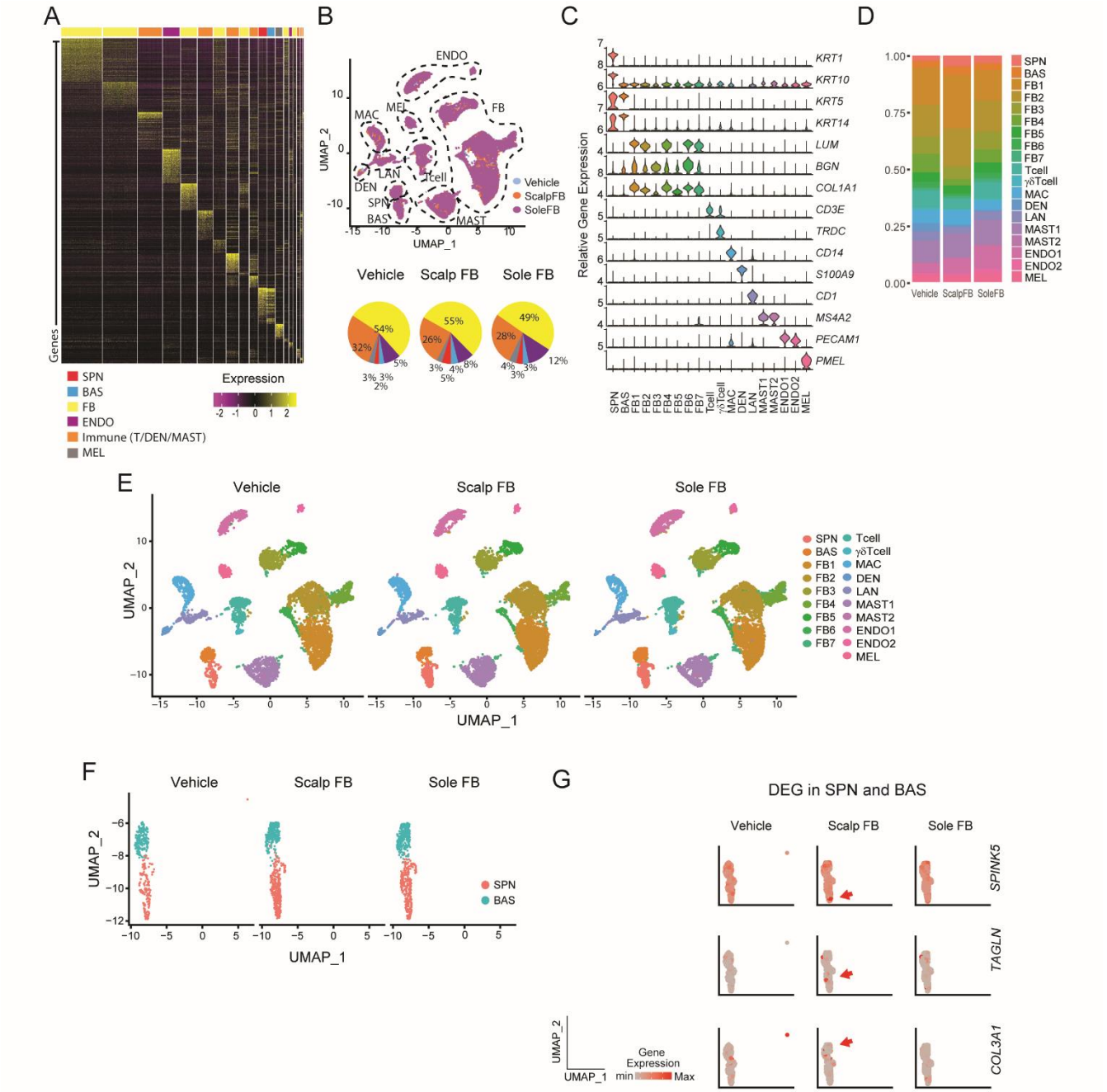


Fig. S16. Bioinformatics with scRNAseq from the 17-month post-injection time point. (A) Heat map of top 200 gene expressions. (B) UMAP of clusters and pie charts of each cluster. (C) Violin plots of marker gene expression in each cluster. (D) Frequency diagram of cell clusters. (E) UMAP plots of injection sites. (F) UMAP plot of keratinocytes. (G) Dot plot of DEG in keratinocytes.

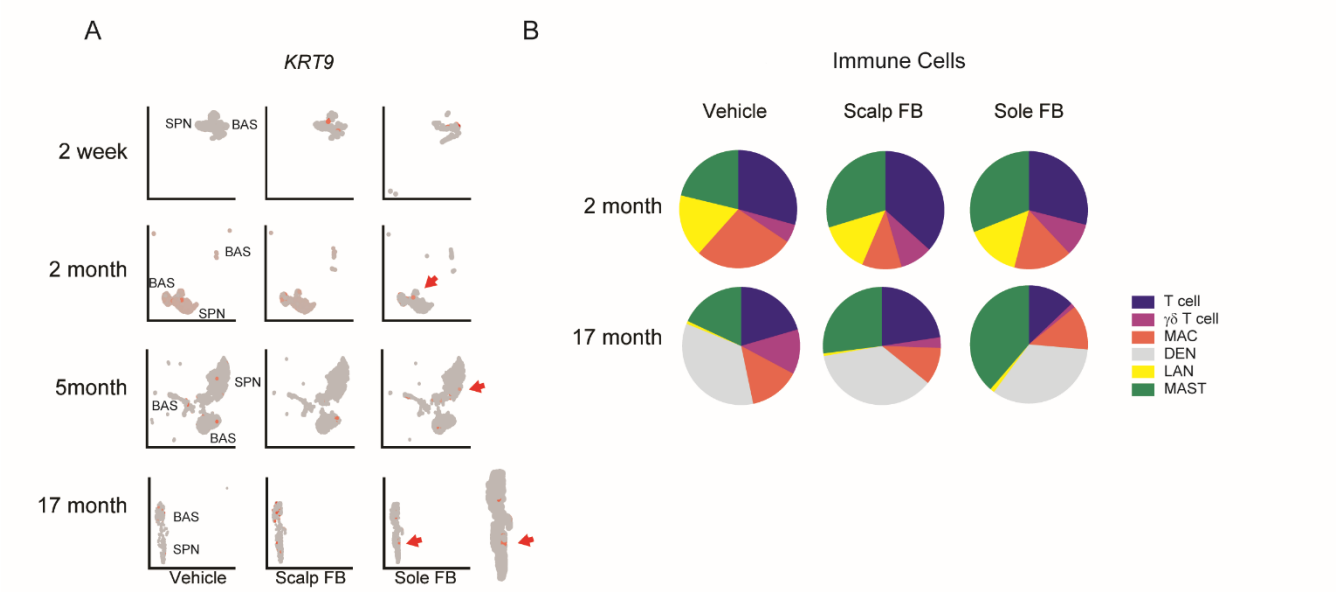


Fig. S17. *KRT9* expression and immune cell composition in post-injection sites from scRNAseq. (A) Dot plots show distribution of KRT9 in the Vehicle, Scalp FB, and Sole FB injection sites. (B) Pie charts illustrate the difference in immune cells among the injection sites at 2- and 17-month post-injection time points.

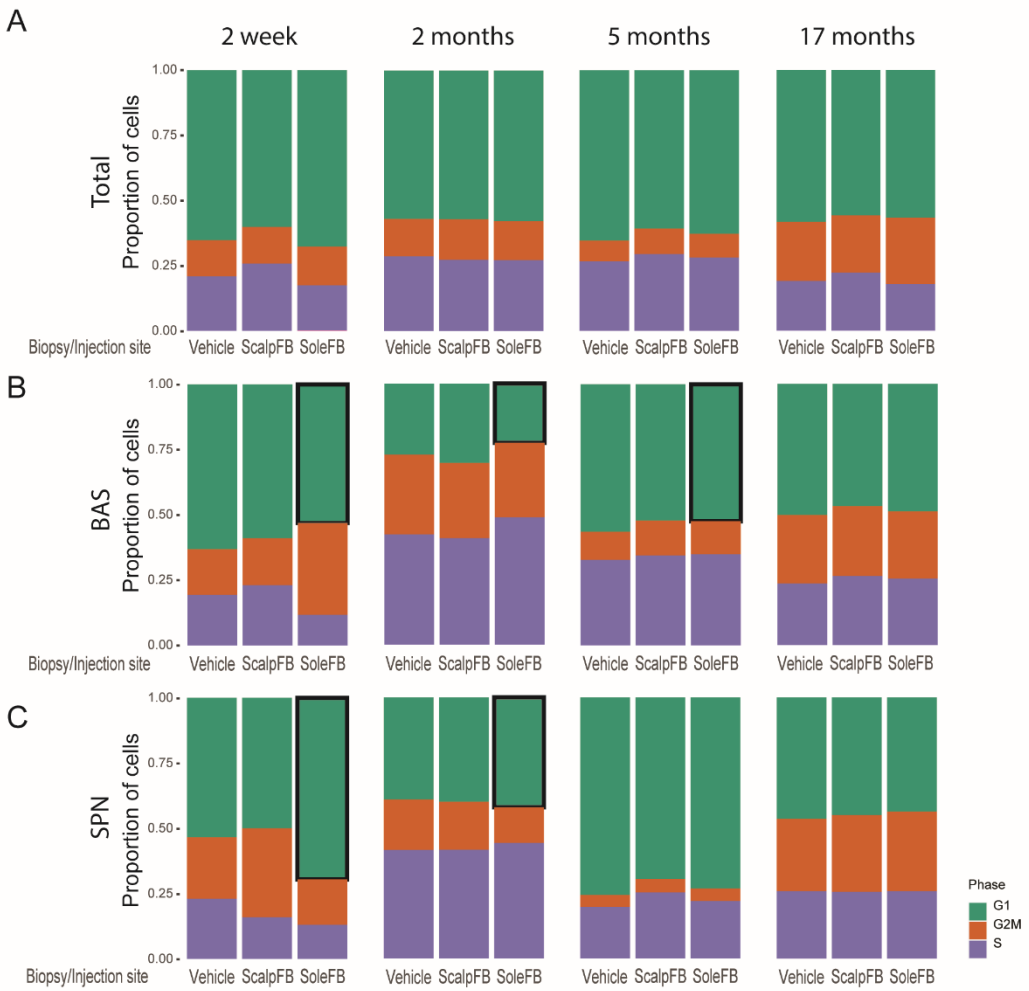


Fig. S18. Proportion of cells in each phase of the cell cycle. (A) Cell cycle proportion of total cells from cell injection sites at different post-injection time points. (B) Cell cycle proportion of BAS clusters from cell injection sites. (C) Cell cycle proportion of SPN clusters from cell injection sites.

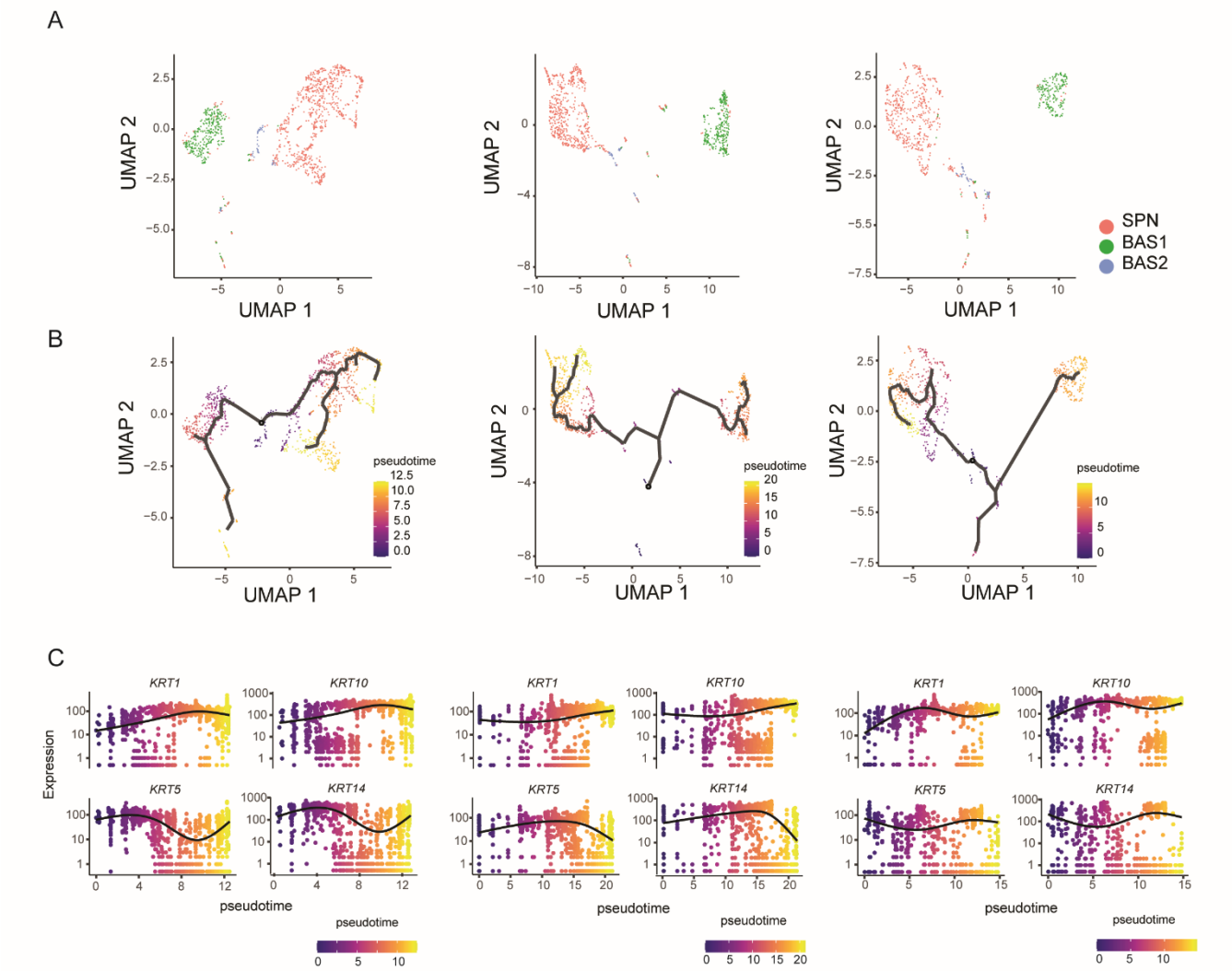


Fig. S19. Pseudotime analysis of keratinocyte clusters at the 2-month post-injection time point. (A) 2D UMAP plot of keratinocytes from Vehicle, Scalp FB, and Sole FB injection sites. (B) Pseudotime analysis of plots from (A). (C) Pseudotime analysis on expression of KRT1/10 and KRT5/14 markers.

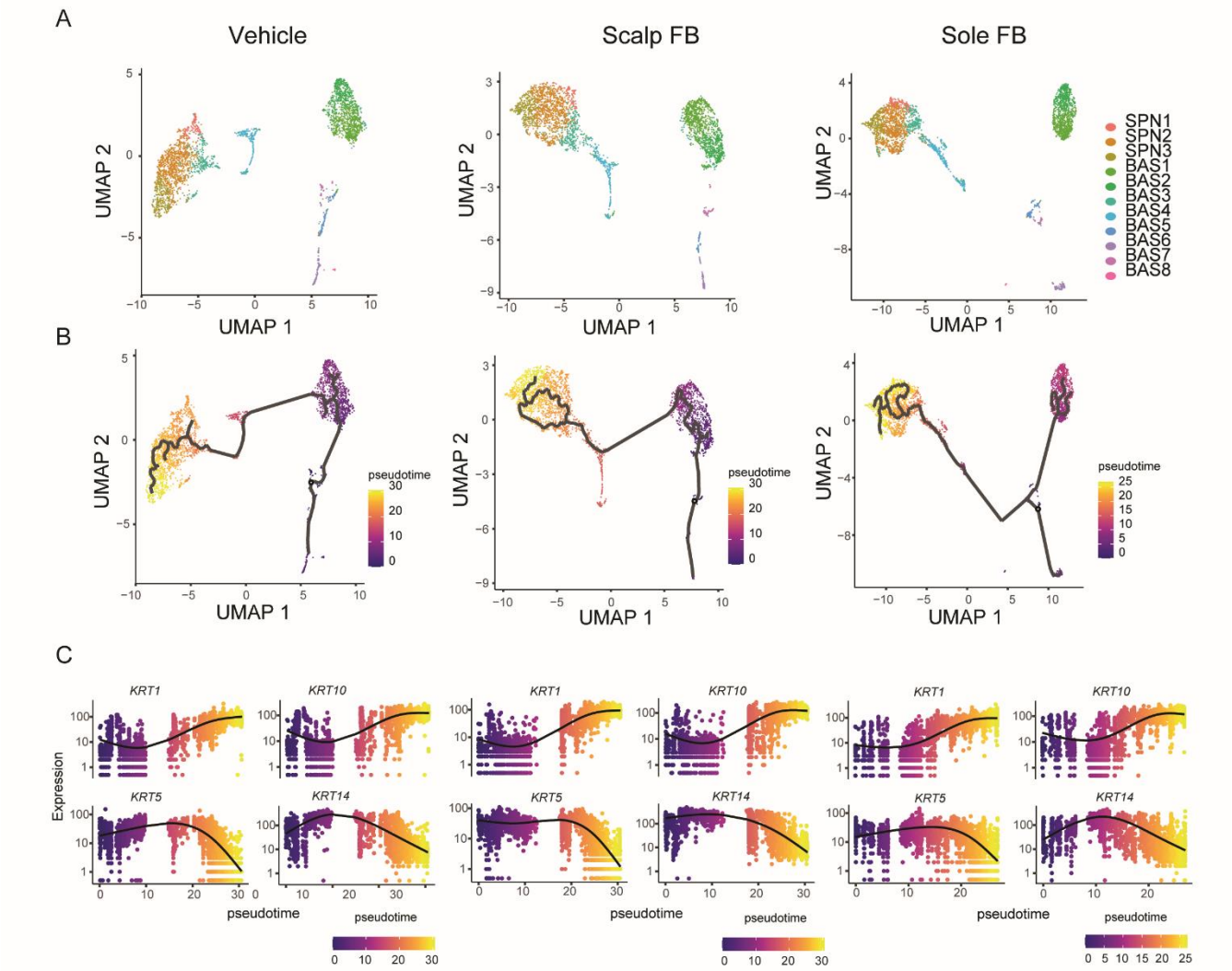


Fig. S20. Pseudotime analysis of keratinocyte clusters at the 5-month post-injection time point. (A) 2D UMAP plot of keratinocytes. (B) Pseudotime analysis. (C) Pseudotime analysis on expression of KRT1/10 and KRT5/14 markers.

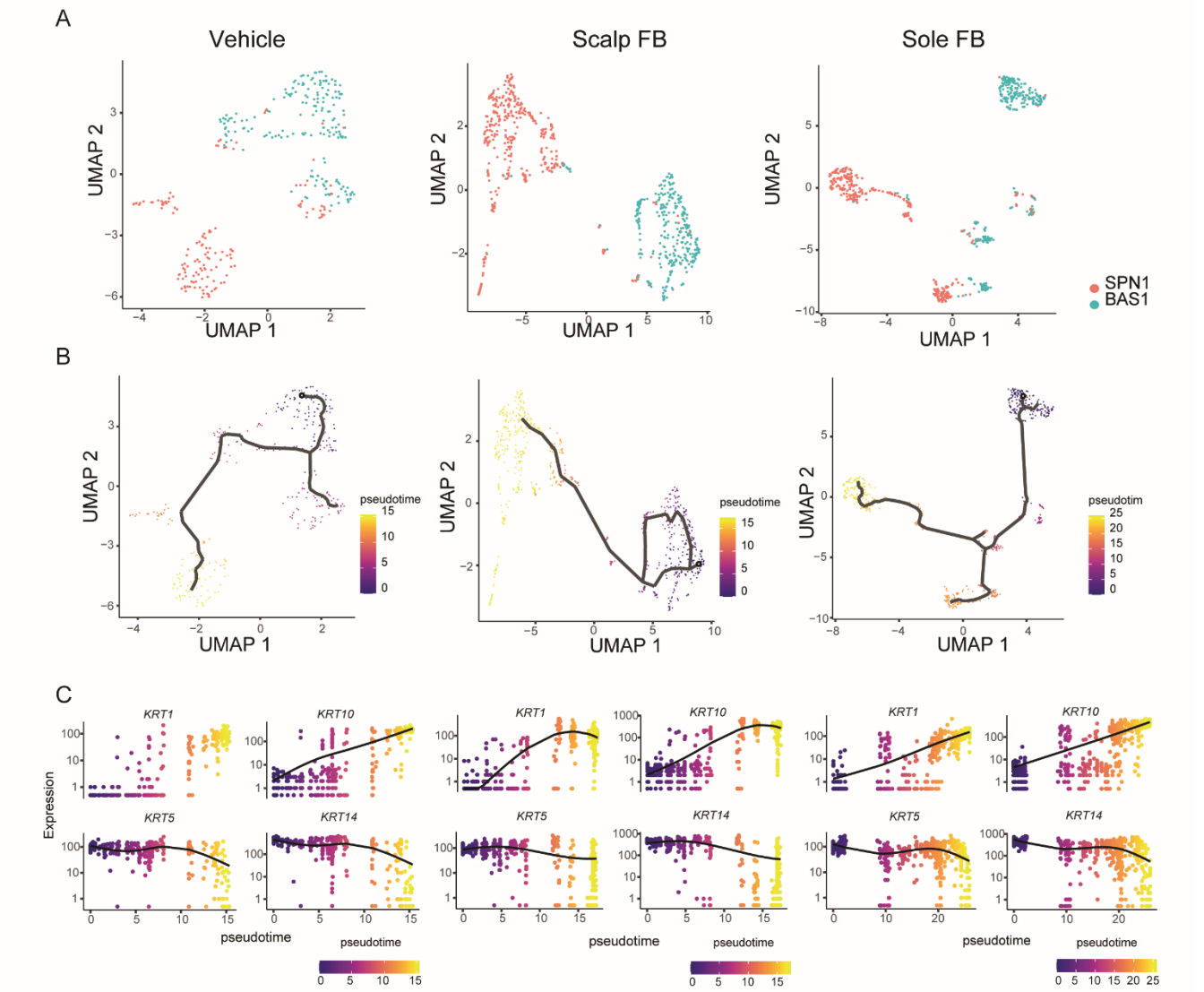


Fig. S21. Pseudotime analysis of keratinocyte clusters at the 17-month post-injection time point. (A) 2D UMAP plot of keratinocytes. (B) Pseudotime analysis. (C) Pseudotime analysis on expression of KRT1/10 and KRT5/14 markers.

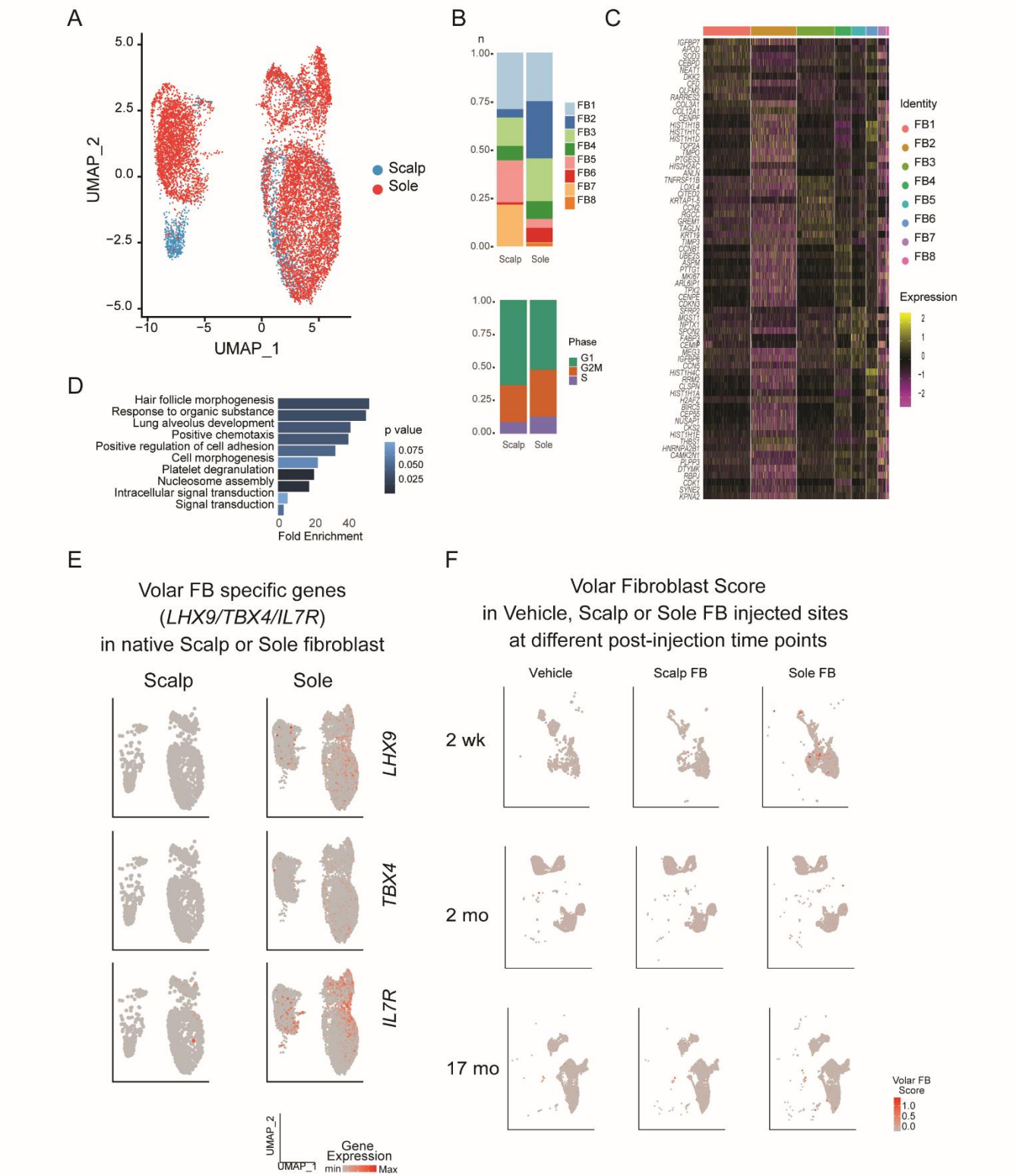


Fig. S22. Bioinformatics with scRNAseq from cultured native scalp and sole fibroblasts. (A) UMAP plot illustrating differences in gene expression between native scalp and sole fibroblasts. (B) Frequency diagram of fibroblast clusters in native scalp and sole fibroblasts, as well as their relative proportion of cell cycle. (C) Heatmap of top 10 genes for each cluster. (D) DEG from in vitro cultured sole fibroblast versus scalp fibroblast with padj <

0.05, listed in table S16 (sole fibroblast/scalp fibroblast comparison), were further analyzed using DAVID analysis and selected for presentation. (E) UMAP plot which shows LHX9/TBX4/IL7R gene expression in native scalp and sole fibroblasts. (F) Dot plot of volar fibroblast score in vehicle, scalp FB, and sole FB injected sites at the 2-week, 2-month, and 17-month post-injection time points.

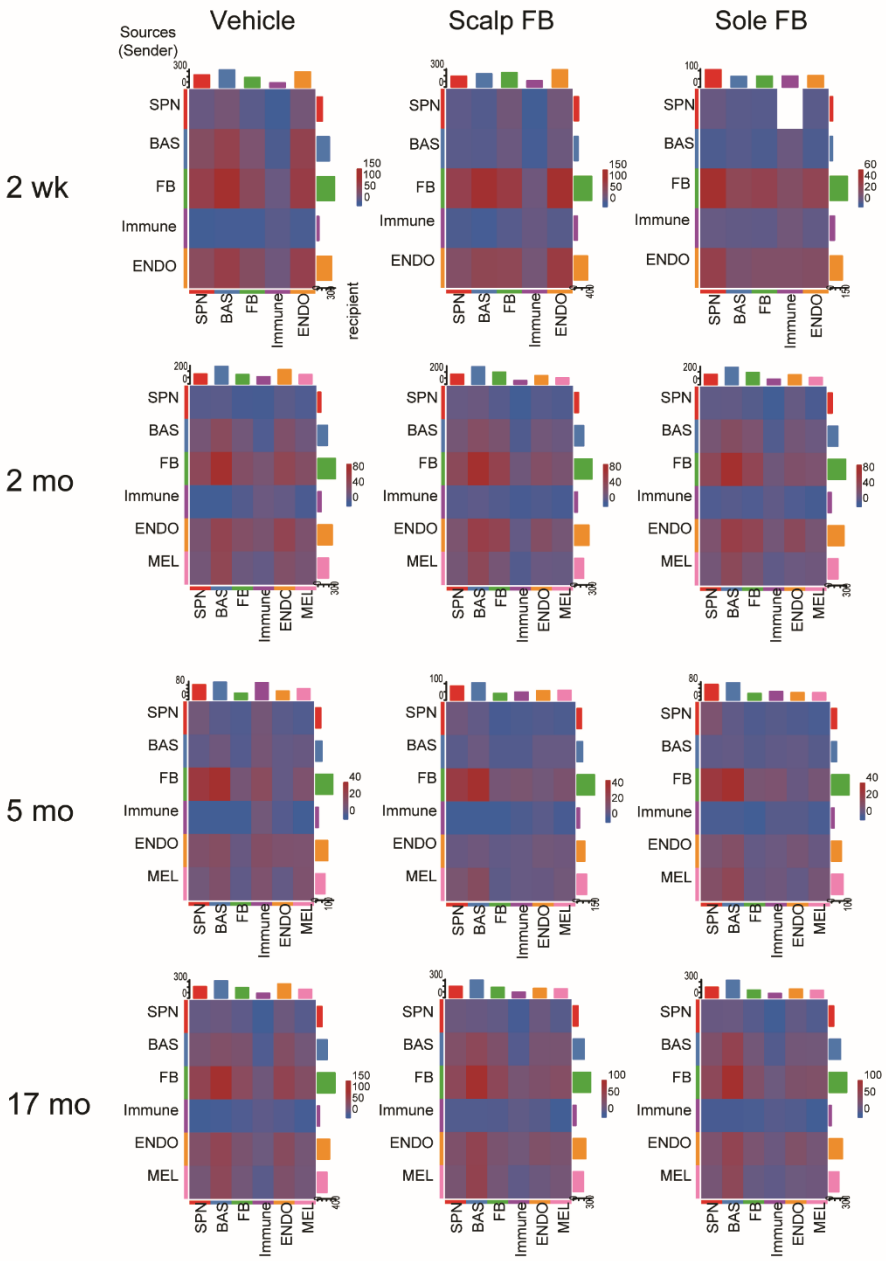


Fig. S23. Heat maps illustrating cell-cell interactions between source (y axis) and recipient (x axis) cell types. Heatmaps represent the magnitude and direction of cell-cell communication present at 2 weeks, 2 months, 5 months, and 17 months post-injection. Cell types sending out signals shown on y axis and cell types receiving signals are shown on x axis.

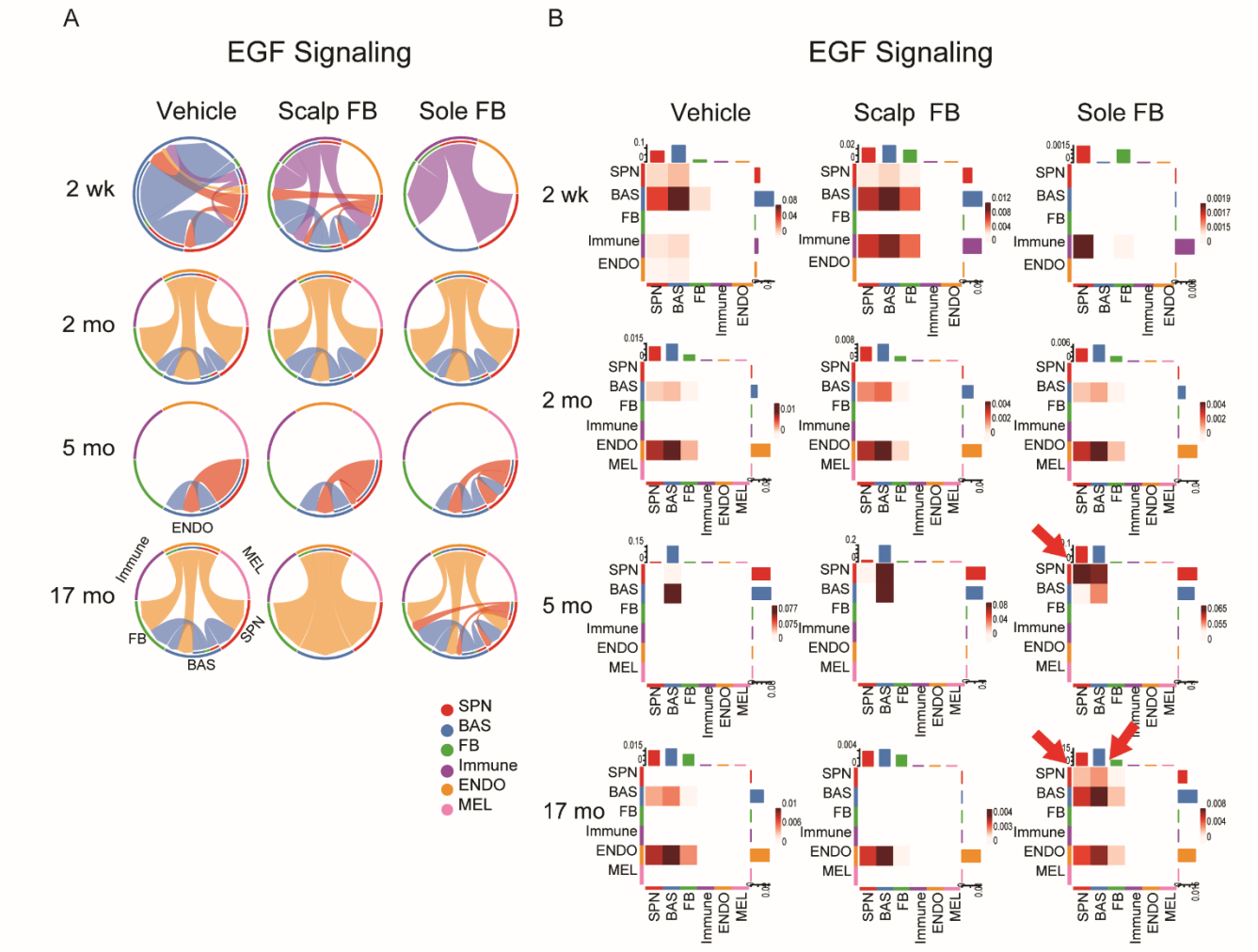


Fig. S24. EGF signaling among cell types. (A) CellChat circle plots representing the magnitude and type of cell-cell communication present at 2 weeks, 2 months, 5 months, and 17 months post-injection. (B) Heat maps depicting cell-cell interactions from the EGF signaling pathway at 2 weeks, 2 months, 5 months, and 17 months post-injection.

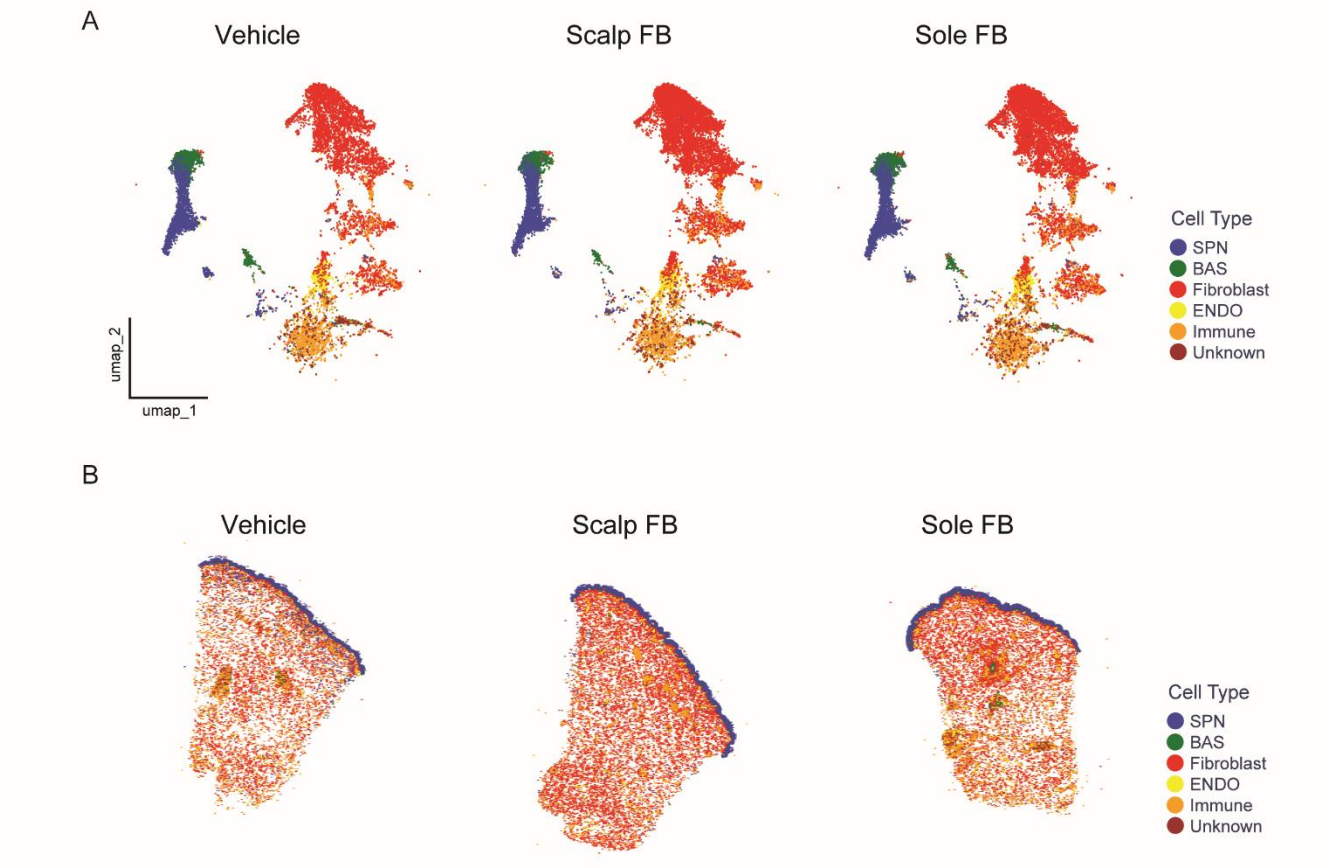
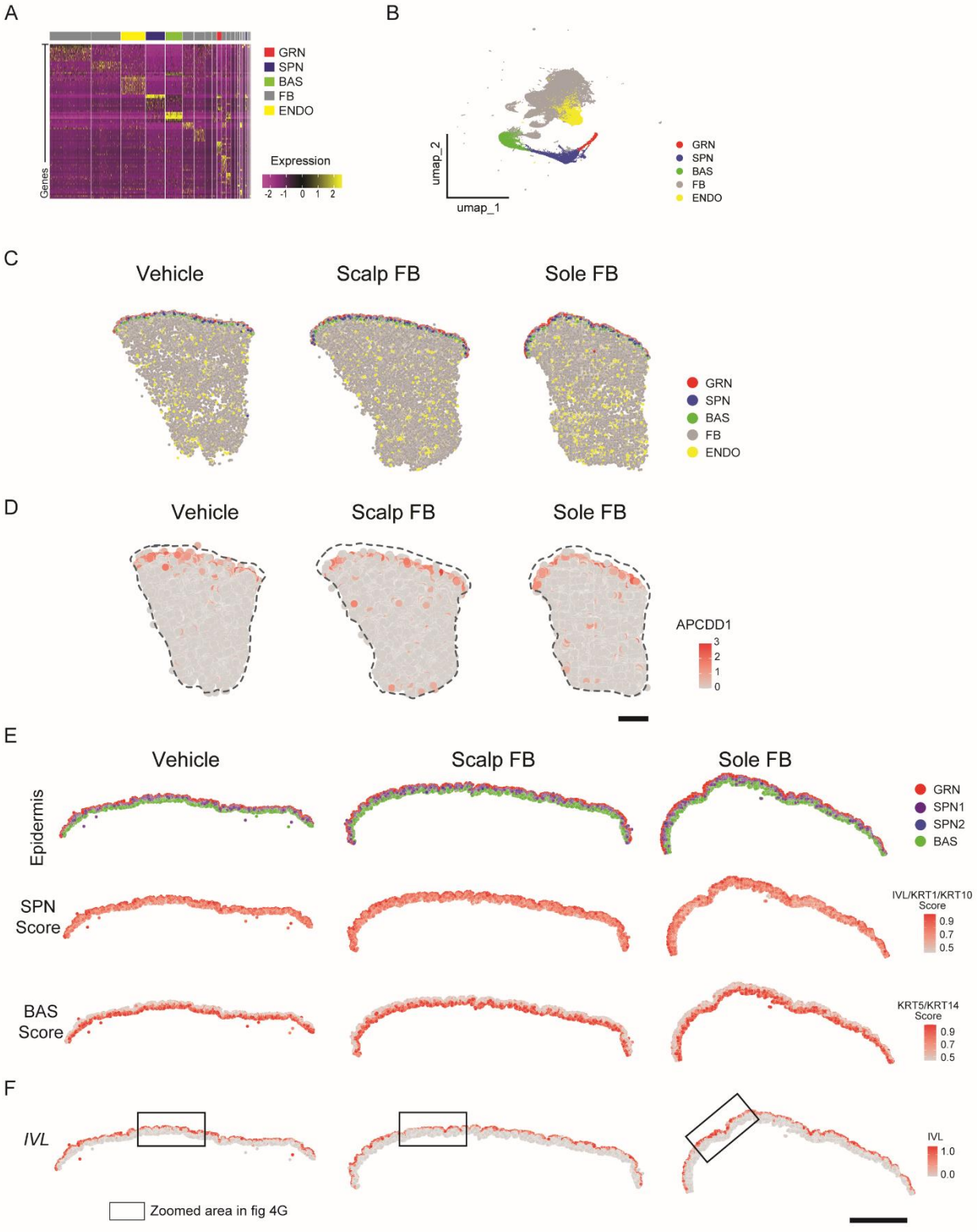


Fig. S25. Cell type identification in Xenium using scSorter and SciPy. Individual cell types are illustrated on UMAP plots (A) and spatial plots (B). Fibroblast densities at injection sites were further calculated and presented in table 2 and fig. 4F.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ENDO
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Sole FB
GRN
SPN
BAS
FB
ENDO
D
Vehicle
Scalp FB
Sole FB
APCDD1
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E
Vehicle
Scalp FB
Sole FB
Epidermis
SPN Score
BAS Score
GRN
SPN1
SPN2
BAS
IVL/KRT1/KRT10 Score
0.9
0.7
0.5
KRT5/KRT14 Score
0.9
0.7
0.5
F
IVL
IVL
1.0
0.0
Zoomed area in fig 4G

Fig. S26. Analysis of Xenium data using Seurat. (A) Heatmap of the top 100 genes. (B) UMAP plot showing the cluster grouping based on gene expression. (C) Spatial plots illustrating individual cells. (D) Spatial plots showed APCDD1 expression on fibroblasts at papillary regions. Scale bar = 1 mm. (E). Epidermal layers were isolated for further analysis. Spatial plots at injection sites illustrate SPN scores using *IVL*, *KRT1* and *KRT10* as well as BAS scores using *KRT5* and *KRT14*. (F) *IVL* expression depicted in the epidermal layers at injection sites. Zoomed-in views of boxed regions are shown in fig. 4G. Scale bar = 1mm.

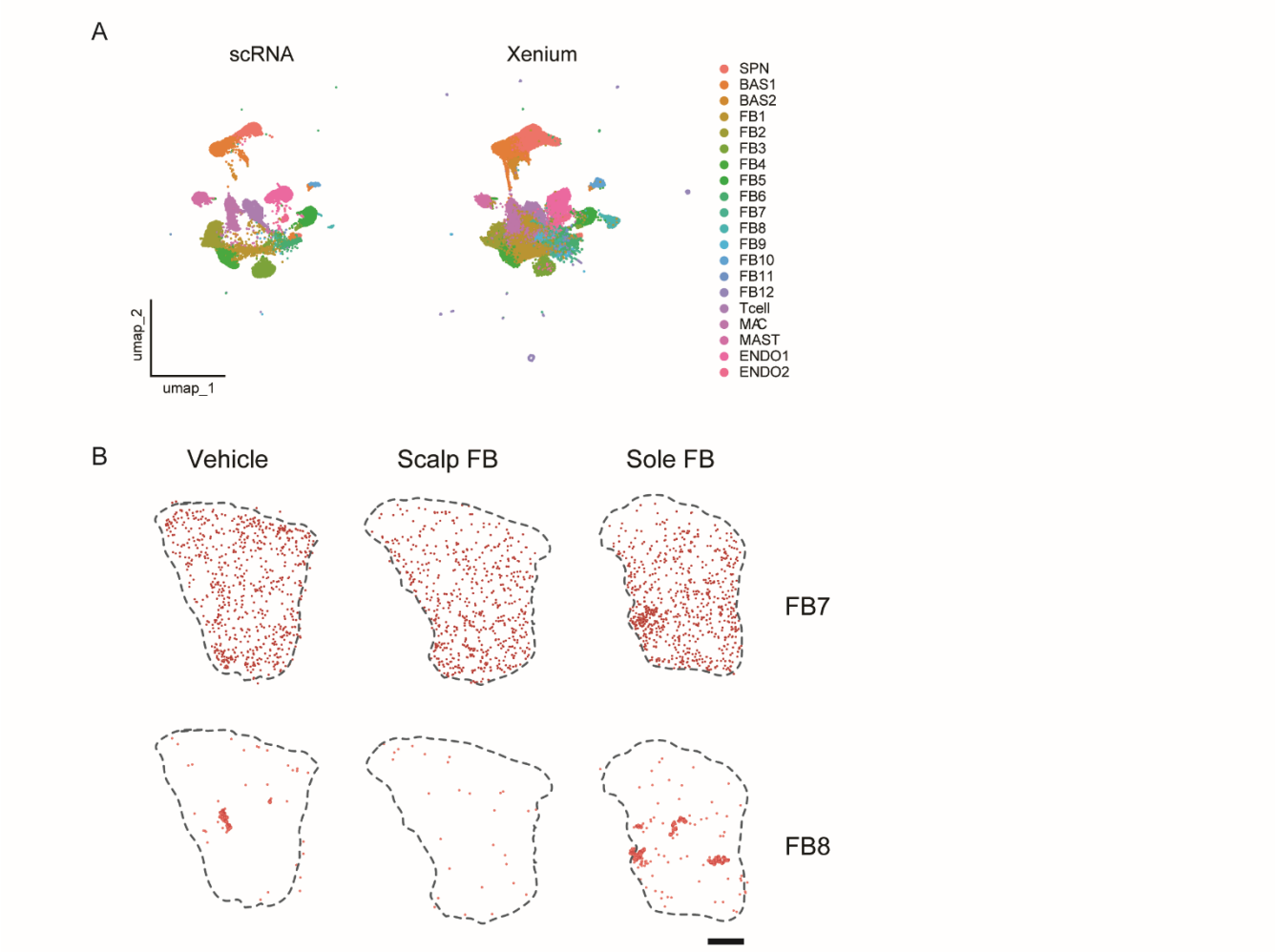

Fig. S27. Integration of Xenium and scRNA data at 2 months post-injection time point. (A) UMAP plots of integrated Xenium and scRNA data showed that similar cluster distribution. (B) Spatial plots of volar and HOX gene-positive clusters (detailed top 10 genes listed in table S21) showed that FB8 cluster is dominated in sole fibroblast injection site. Scale bar = 1 mm.

Legend for table S1. DEG from bulk RNAseq in fibroblasts + pressure versus control fibroblasts. DEG from bulk RNAseq in scalp fibroblasts + pressure vs. control scalp fibroblasts and sole fibroblasts + vs. control sole fibroblasts were identified. Table S1 is provided as an Excel file.

Table S2. Comparison of DEG between sole and scalp fibroblast by dynamic pressure treatment. Top25 upregulated DEG in sole fibroblast + pressure vs. control sole fibroblast were compared with DEG in scalp fibroblast + pressure vs. control scalp fibroblast, and vice versa.

	Gene ID
Top25 upregulated DF genes in Sole FB + pressure / Sole FB	*AC004264.1, AC025259.3, ARC, ATF3, C11orf96, CSRNP1, CXCL2, CXCL3, DUSP1, DUSP2, EGR1, EGR2, EGR3, EGR4, FOS, FOSB, HBEGF, IL6, JUNB, LIF, NFKBIZ, NR4A1, NR4A2, PTGS2, TCIM*
Unique in Top 25 upregulate DF in Sole FB + pressure / Sole FB	*AC004264.1, AC025259.3, ARC, ATF3, C11orf96, CSRNP1, CXCL2, CXCL3, DUSP1, DUSP2, EGR1, EGR2, EGR4, HBEGF, IL6, JUNB, LIF, NFKBIZ, NR4A1, TCIM*
Common in Sole FB+Pressure / Sole FB AND DF genes in scalp FB + pressure / scalp FB	*EGR3, FOS, FOSB, NR4A2, PTGS2*

	Gene ID
Top25 upregulated DF genes in Scalp FB + pressure / Scalp FB	*AIF1L, AP000892.3, DUSP1, DUSP2, EGR1, EGR2, EGR3, FOS, FOSB, IER2, IER3, IGSF10, JUNB, KLF2, NFKBIZ, NR4A1, OMD, PIM1, PTGS2, RGS16, STUM, TPRG1, TRIL, ZC3H12A, ZFP36*
Unique in Top 25 upregulate DF in Scalp FB + pressure / Scalp FB	*AIF1L, AP000892.3, IGSF10, OMD, RGS16, STUM, TPRG1, TRIL*
Common in Scalp FB + Pressure / Scalp FB AND DF genes in Sole FB + pressure / Sole FB	*DUSP1, DUSP2, EGR1, EGR2, EGR3, FOS, FOSB, IER2, IER3, JUNB, KLF2, NFKBIZ, NR4A1, PIM1, PTGS2, ZC3H12A, ZFP36*

Legend for table S3. DEG from bulk RNAseq in Sole fibroblast versus Scalp fibroblast in the absence or presence of pressure. DEG from bulk RNAseq in sole fibroblasts vs. scalp fibroblasts and sole fibroblast + pressure vs. scalp fibroblast + pressure were identified. Table S3 is provided as an Excel file.

Table S4. Inclusion and exclusion criteria applied to the study participants. Detailed criteria applied to the study participants outlined the inclusion and exclusion parameters that were used to determine eligibility for the study.

Inclusion Criteria

- Aged 18-75 years
- Be willing and able to comply with the scheduled visits, biopsy/injection procedures, wound care instructions treatment plan, and other study procedures for the duration of the study
- Able to undergo administration of the study material. This is determined by the investigator and based on evidence of no clinically significant abnormality from laboratory tests conducted up to 14 days prior to the start of the study
- For females of childbearing potential,
    - Have a negative pregnancy test at screening
    - Agree to not become pregnant or breastfeed for the period of the study through 1 month after completion of the study
    - Be willing to use a reliable form of contraception during the study
- Have healthy skin as determined by the investigatory or study Nurse Practitioner
- Able to provide informed consent

Exclusion Criteria

- Received any investigational drug within 30 days prior to study entry
- Have a history of allergies to any study materials, including local anesthetic, dimethyl sulfoxide, human albumin, bovine constituents, or hetastarch
- Be pregnant, lactating, or trying to become pregnant
- Have a history of keloid formation
- Have an active, nonhealing wound
- Have a significant medical history that the investigator feels is not safe for study participation (for example, some forms of autoimmune conditions, metastatic cancer, infectious diseases such as HIV, HTLV I/II, Hepatitis B, and Hepatitis C). If a biopsy is infected with a pathogen that is not allowed to enter the cell therapy core, then the individual cannot participate.
- Have an autoimmune disease that affects the skin, such as lupus
- Have a skin disease that the investigator feels is not safe for study participation (e.g., extreme and active eczema, psoriasis, lichen planus)
- Have a diagnosis of uncontrolled diabetes
- Be an active smoker during the study
- Use chronic immunosuppressive therapies, such as oral steroids, or chronic topical steroids in the area of investigation
- Have bovine or meat sensitivity or severe allergies manifested by anaphylaxis to any product
- Have a bleeding disorder
- If receiving narrowband UVB,
    - Have a history of skin cancer in the area of skin which would receive irradiation
    - Have a history of photosensitive skin conditions (e.g. lupus, porphyria, dermatomyositis)

Legend for table S5. Side effects of cell injections. Details of observed side effects following Immediate and one-week post-injection cell injection were documented. Table S5 is provided as an Excel file.

Legend for table S6. DEG from bulk RNAseq in single injection (1x). DEG from bulk RNAseq at the scalp fibroblasts injection site (Scalp FB) vs. vehicle injection site (Vehicle) and sole fibroblasts injection site (Sole FB) vs. vehicle injection site (Vehicle) were identified. Table S6 is provided as an Excel file.

Legend for table S7. DEG from bulk RNAseq in fractionated multiple injections (3x). DEG from bulk RNAseq at the scalp fibroblasts injection site (Scalp FB) vs. vehicle injection site (Vehicle) and sole fibroblasts injection site (Sole FB) vs. vehicle injection site (Vehicle) were identified. Table S7 is provided as an Excel file.

Legend for table S8. Top genes for cluster identification in 2 weeks post-injection scRNAseq data. Top genes were identified from the integrated Seurat object comprising Vehicle, Scalp FB and Sole FB from scRNAseq data at 2 weeks post-injection time point. Table S8 is provided as an Excel file.

Legend for table S9. Top genes for cluster identification in 2 months post-injection scRNAseq data. Top genes were identified from the integrated Seurat object comprising Vehicle, Scalp FB and Sole FB from scRNAseq data at 2 months post-injection time point. Table S9 is provided as an Excel file.

Legend for table S10. Top genes for cluster identification in 5 months post-injection scRNAseq data. Top genes were identified from the integrated Seurat object comprising Vehicle, Scalp FB and Sole FB from scRNAseq data at 5 months-post injection time point scRNAseq data. Table S10 is provided as an Excel file.

Legend for table S11. Top genes for cluster identification in 17 months post-injection scRNAseq data. Top genes were identified from the integrated Seurat object comprising Vehicle, Scalp FB and Sole FB from scRNAseq data at 17 months post-injection time point. Table S11 is provided as an Excel file.

Legend for table S12. DEG of SPN and BAS in 2 weeks post-injection scRNAseq data. DEG from scRNAseq at the scalp fibroblasts injection site (Scalp FB) vs. vehicle injection site (Vehicle) and sole fibroblasts injection site (Sole FB) vs. vehicle injection site (Vehicle) in SPN and BAS populations in 2 weeks post-injection timepoint were identified. Table S12 is provided as an Excel file.

Legend for table S13. DEG of SPN and BAS in 2 months post-injection scRNAseq data. DEG from scRNAseq at the scalp fibroblasts injection site (Scalp FB) vs. vehicle injection site (Vehicle) and sole fibroblasts injection site (Sole FB) vs. vehicle injection site (Vehicle) in SPN and BAS populations in 2 months post-injection timepoint were identified. Table S13 is provided as an Excel file.

Legend for table S14. DEG of SPN and BAS in 5 months post-injection scRNAseq data. DEG from scRNAseq at the scalp fibroblasts injection site (Scalp FB) vs. vehicle injection site (Vehicle) and sole fibroblasts injection site (Sole FB) vs. vehicle injection site (Vehicle) in SPN and BAS populations in 5 months post-injection timepoint were identified. Table S14 is provided as an Excel file.

Legend for table S15. DEG of SPN and BAS in 17 months post-injection scRNAseq data. DEG from scRNAseq at the scalp fibroblasts injection site (Scalp FB) vs. vehicle injection site (Vehicle) and sole fibroblasts injection site

(Sole FB) vs. vehicle injection site (Vehicle) in SPN and BAS populations in 17 months post-injection timepoint were identified. Table S15 is provided as an Excel file.

Legend for table S16. DEG of in vitro cultured fibroblasts from scRNAseq data. DEG from scRNAseq at the sole fibroblasts vs. scalp fibroblasts were identified. The entire fibroblast cluster from sole and scalp fibroblasts were compared as a single population and each individual fibroblast sub-population from sole and scalp fibroblasts was also compared. Table S16 is provided as an Excel file.

Legend for table S17. Top 100 dermal genes in volar versus non-volar skin microarray data (GSE39266). DEG from scRNAseq at the sole fibroblasts vs. scalp fibroblasts were identified. The entire fibroblast cluster from sole and scalp fibroblasts were compared as a single population and each individual fibroblast sub-population from sole and scalp fibroblasts was also compared. Table S17 is provided as an Excel file.

Table S18. Xenium custom panel. In addition to the 350 target genes on human multi-tissue and cancer panel, 34 target genes were used in Xenium detection.

Name	ID	Name	ID
LHX9	ENSG00000143355	*NOTCH1*	ENSG00000148400
TBX4	ENSG00000121075	*FGF7*	ENSG00000140285
NRG1	ENSG00000157168	*LUM*	ENSG00000139329
HOXA10	ENSG00000253293	*APOE*	ENSG00000130203
HOXA11	ENSG00000005073	*APOD*	ENSG00000189058
COL1A1	ENSG00000108821	*POSTN*	ENSG00000133110
COL3A1	ENSG00000168542	*KRT9*	ENSG00000171403
CTSC	ENSG00000109861	*KRT16*	ENSG00000186832
MAP1B	ENSG00000131711	*GJB2*	ENSG00000165474
MGST1	ENSG00000008394	*GJB6*	ENSG00000121742
KRT1	ENSG00000167768	*ENTPD1*	ENSG00000138185
KRT10	ENSG00000186395	*CD36*	ENSG00000135218
KRT5	ENSG00000186081	*ENG*	ENSG00000106991
KRT14	ENSG00000186847	*IVL*	ENSG00000163207
KRT17	ENSG00000128422	*FOSL1*	ENSG00000175592
KRT19	ENSG00000171345	*SOX9*	ENSG00000125398
RBPJ	ENSG00000168214	*GRHL3*	ENSG00000158055

Table S19. Marker genes used in scSorter with Xenium data at 2 months post-injection time point. Marker genes were analyzed using scSorter to identify specific cell types in Xenium data at 2 months post-injection time point.

Cell type	Marker gene
Fibroblast	*COL1A1*
Fibroblast	*COL3A1*
Fibroblast	*LUM*
SPN	*KRT1*
SPN	*KRT10*
BAS	*KRT14*
BAS	*KRT5*
ENDO	*PECAM1*
Tcell	*CD3E*
Tcell	*CD69*
MAC	*CD14*
LAN	*CD1C*
MAST	*MS4A2*

Legend for table S20. Top genes for cluster identification in 2 months post injection Xenium data. Top genes were identified from Xenium data at 2 months post injection time point. Table S20 is provided as an Excel file.

Legend for table S21. Top genes for cluster identification from integrated scRNAseq and Xenium data at 2 months post-injection time point. Top genes were identified from the integrated Seurat object comprising scRNAseq and Xenium data at 2 months post-injection time point. Table S21 is provided as an Excel file.

Titles for movies S1-S5:
Movie S1. Slingshot movement of scalp fibroblast in 0 kPa.
Movie S2. Slingshot movement of scalp fibroblast in 100 kPa.
Movie S3. Slingshot movement of sole fibroblast in 0 kPa.
Movie S4. Slingshot movement of sole fibroblast in 100 kPa.
Movie S5. *in vitro* dynamic oscillating pressure system