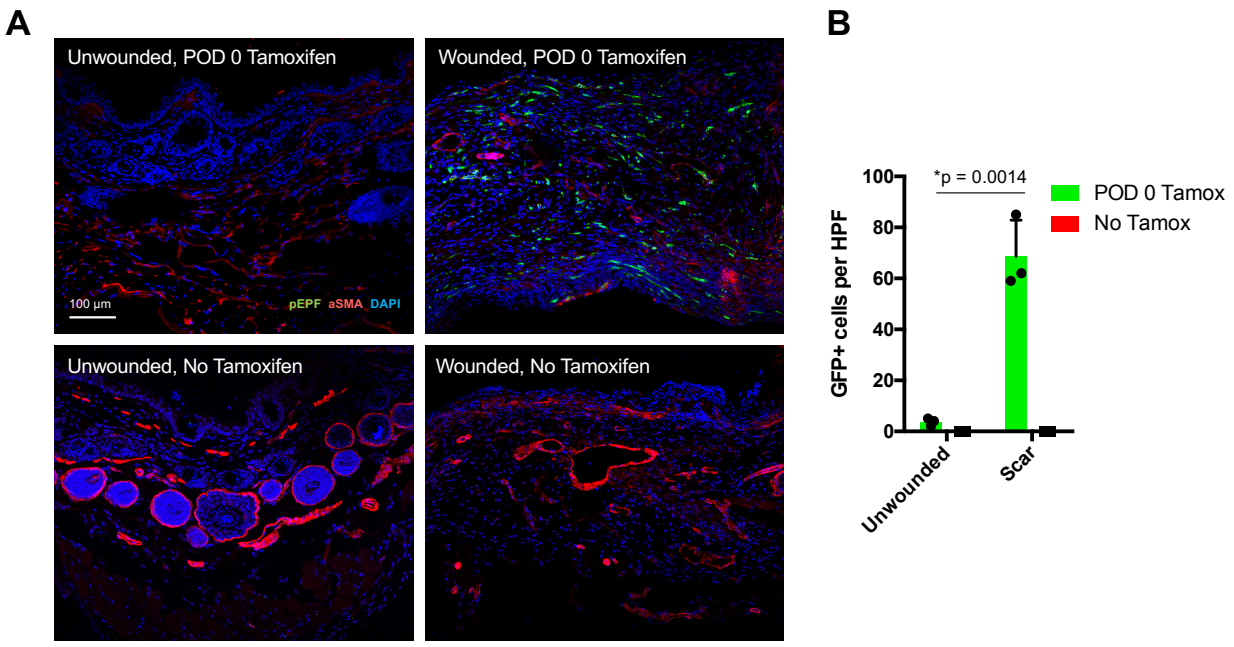


Figure S1: Tamoxifen dosing controls. (A) Immunofluorescent histology of unwounded skin and scars in *En-1*$^{Cre\text{-}ERT}$*;Ai6* mice that received tamoxifen at the time of wounding (top row) or no tamoxifen (bottom row). **(B)** Quantification of GFP+ cells (pEPFs) per 20x HPF. Unwounded vs. scar, POD 0 tamoxifen **P* = 0.0014; no tamoxifen control not significant.

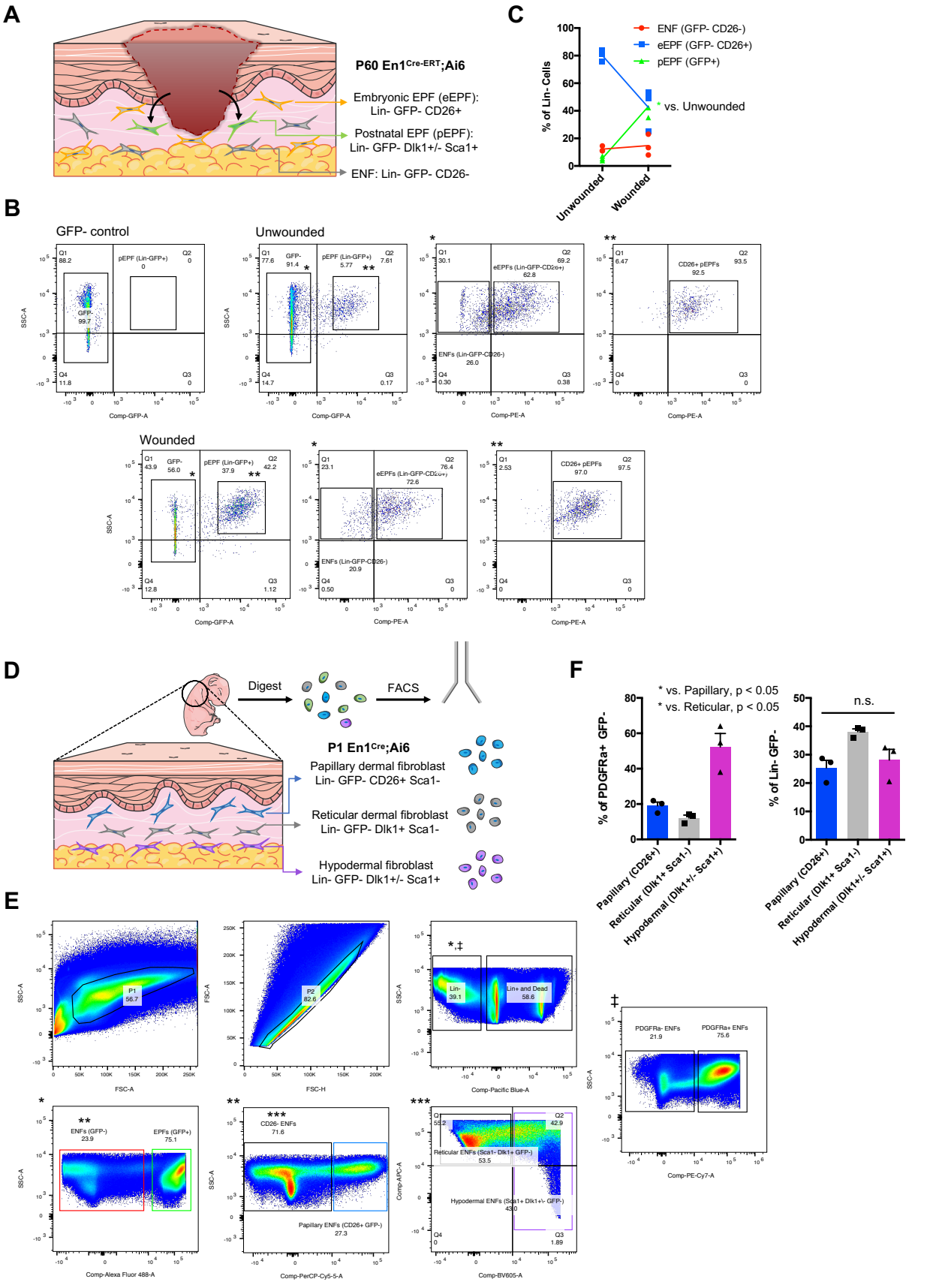

A
P60 En1Cre-ERT;Ai6
Embryonic EPF (eEPF):
Lin- GFP- CD26+
Postnatal EPF (pEPF):
Lin- GFP- Dlk1+/- Sca1+
ENF: Lin- GFP- CD26-
C
ENF (GFP- CD26-)
eEPF (GFP- CD26+)
pEPF (GFP+)
% of Lin- Cells
* vs. Unwounded
Unwounded
Wounded
B
GFP- control
Unwounded
Wounded
SSC-A
Comp-GFP-A
Comp-PE-A
D
Digest
FACS
P1 En1Cre;Ai6
Papillary dermal fibroblast
Lin- GFP- CD26+ Sca1-
Reticular dermal fibroblast
Lin- GFP- Dlk1+ Sca1-
Hypodermal fibroblast
Lin- GFP- Dlk1+/- Sca1+
F
* vs. Papillary, p < 0.05
* vs. Reticular, p < 0.05
n.s.
% of PDGFRa+ GFP-
% of Lin- GFP-
Papillary (CD26+)
Reticular (Dlk1+ Sca1-)
Hypodermal (Dlk1+/- Sca1+)
E
SSC-A
FSC-A
FSC-H
Comp-Pacific Blue-A
Comp-Alexa Fluor 488-A
Comp-PerCP-Cy5-5-A
Comp-APC-A
Comp-BV605-A
Comp-PE-Cy7-A

Figure S2: FACS strategies to isolate fibroblast subtypes. (A) Strategy for isolating ENFs (Lin^- GFP^- $CD26^-$), eEPFs (Lin^- GFP^- $CD26^+$), and pEPFs (Lin^- GFP^+) from tamoxifen-induced *En-1*$^{Cre-ERT}$*;Ai6* dorsal skin and excisional wounds. **(B)** Representative FACS plots for GFP- control (left), unwounded skin (right) and wounds (bottom). *, ** indicate gated cell populations carried over into subsequent plots. **(C)** Quantification of relative proportion of fibroblasts (Lin^-) represented by ENFs (red), eEPFs (blue), and pEPFs (green) in unwounded skin vs. healed wounds (POD 14). Points represent biological replicates; N = 3 biological replicates, each containing pooled cells from 4 mice (2 wounds/mouse). Unwounded vs. wounded: eEPFs, $*P = 0.0559$; pEPFs, $*P = 0.0204$; ENFs, $P = 0.6433$. **(D)** Schematic for FACS isolation of papillary, reticular, and hypodermal fibroblasts from *En-1*^{Cre}*;Ai6* dorsal skin based on previously reported surface markers. **(E)** Representative FACS plots showing gating strategy for isolating ENFs (Lin^- GFP^-; red box) and EPFs (Lin^- GFP^+; green box), and fractionation of ENF subtypes (papillary, blue box; reticular, gray box; hypodermal, purple box). *, **, ***, and ‡ indicate gated cell populations carried over into subsequent plots. **(F)** Proportion of fibroblasts represented by each ENF subpopulation (papillary, blue; reticular, gray; hypodermal, purple) when fibroblasts are defined as $PDGFRa^+$ cells (left panel) versus Lin^- cells (right panel). N = 3 separate experiments using pooled cells from individual litters. Left: papillary vs. hypodermal $*P = 0.0135$, reticular vs. hypodermal $*P = 0.0067$. Right: all pairwise comparisons $P > 0.05$.

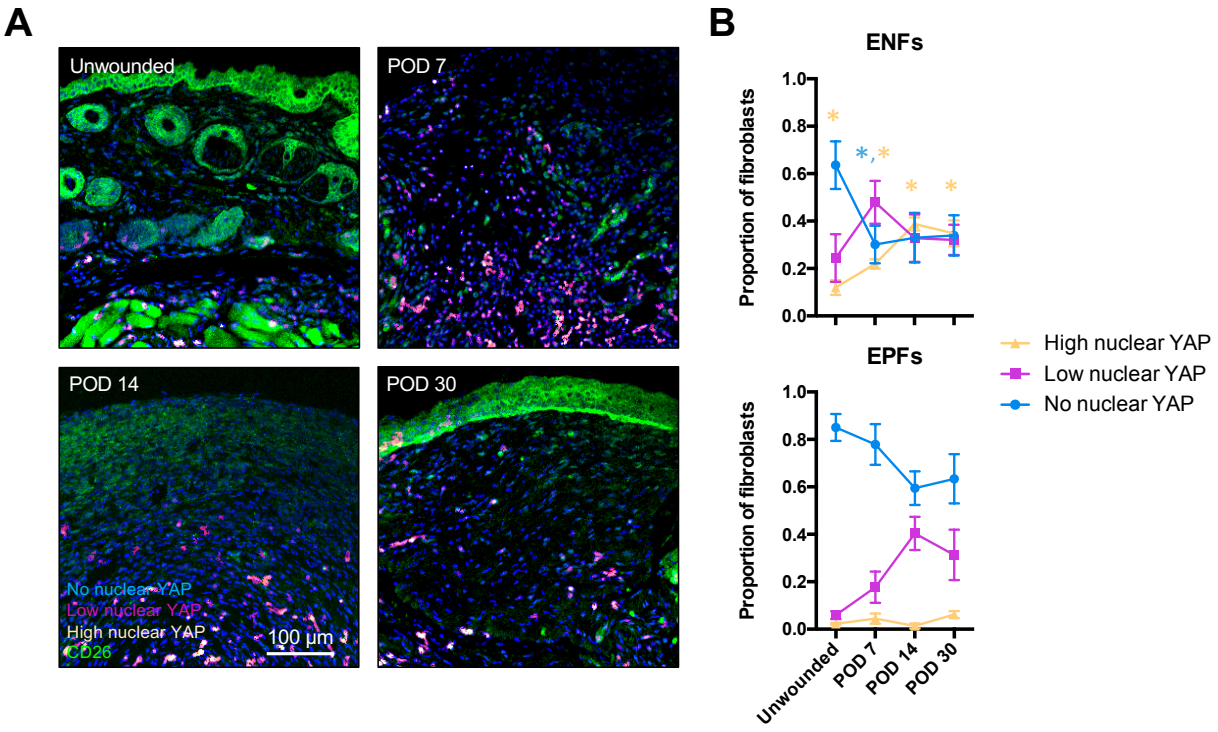
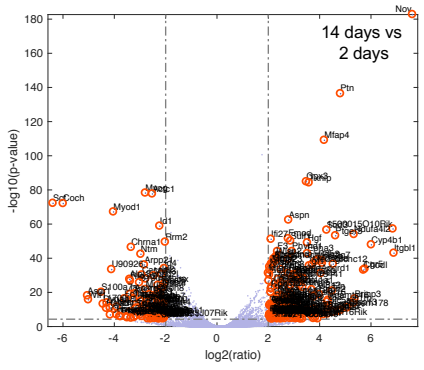
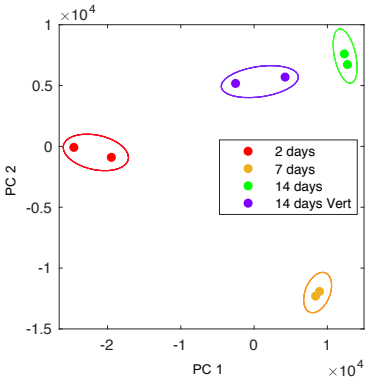


Figure S3: Nuclear YAP localization in ENFs and EPFs. (A) Immunofluorescent histology of excisional wounds at POD 0 (unwounded), 7, 14, and 30. Nuclear localization of YAP in ENFs ($CD26^-$) and EPFs ($CD26^+$) was measured using a custom image processing algorithm. **(B)** Quantification of ENFs and EPFs with no nuclear YAP, low nuclear YAP, and high nuclear YAP. Data points represent the average of at least 3 mice (2 wounds/mouse). ENFs vs EPFs with no nuclear YAP at POD 7, $*P = 0.015$. ENFs vs EPFs with high nuclear YAP at POD 0 ($*P = 0.035$), 7 ($*P = 0.0040$), 14 ($*P = 0.00022$), and 30 ($*P = 0.0022$).

A

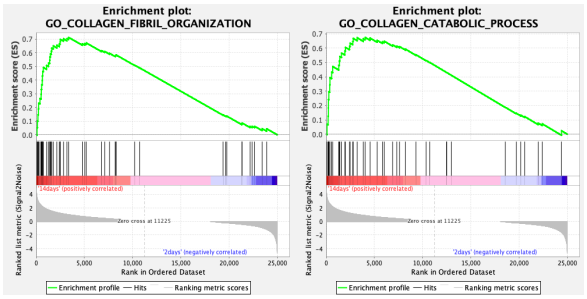


B

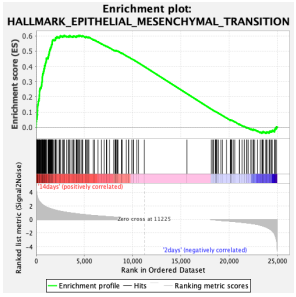


C

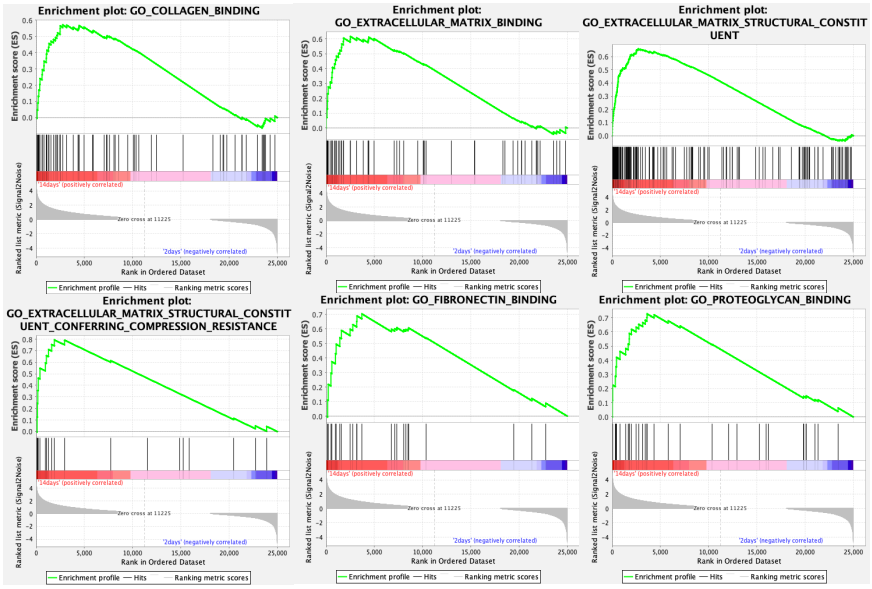
GO-BP::



Hallmarks:



GO-MF:



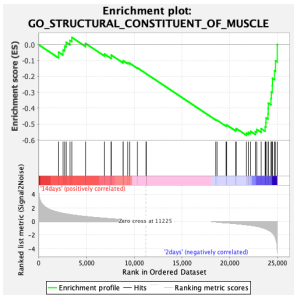


Figure S4: Gene set enrichment analysis for *in vitro* ENFs and pEPFs. (A) Volcano plot of 920 differentially expressed genes between ENFs cultured for 2 or 14 days on TCPS. **(B)** Principal component analysis (PCA) of RNA-seq data from cultured ENFs at different timepoints, with and without Verteporfin treatment. Clusters for each timepoint and condition are indicated by ovals. **(C)** Normalized RNA-seq counts for ENFs ($mTomato^+$) cultured on TCPS for 2 days (remain as ENFs) or 14 days (activate *Engrailed-1*; GFP^+) were analyzed for enrichment in the Gene Ontology Biological Process, Gene Ontology Molecular Function, and Hallmark databases. Activation of *Engrailed-1* was associated with the loss of "muscle development" identity and the gain of a pro-fibrotic identity, as inferred by enrichment for a variety of ECM-related terms at 14 days.

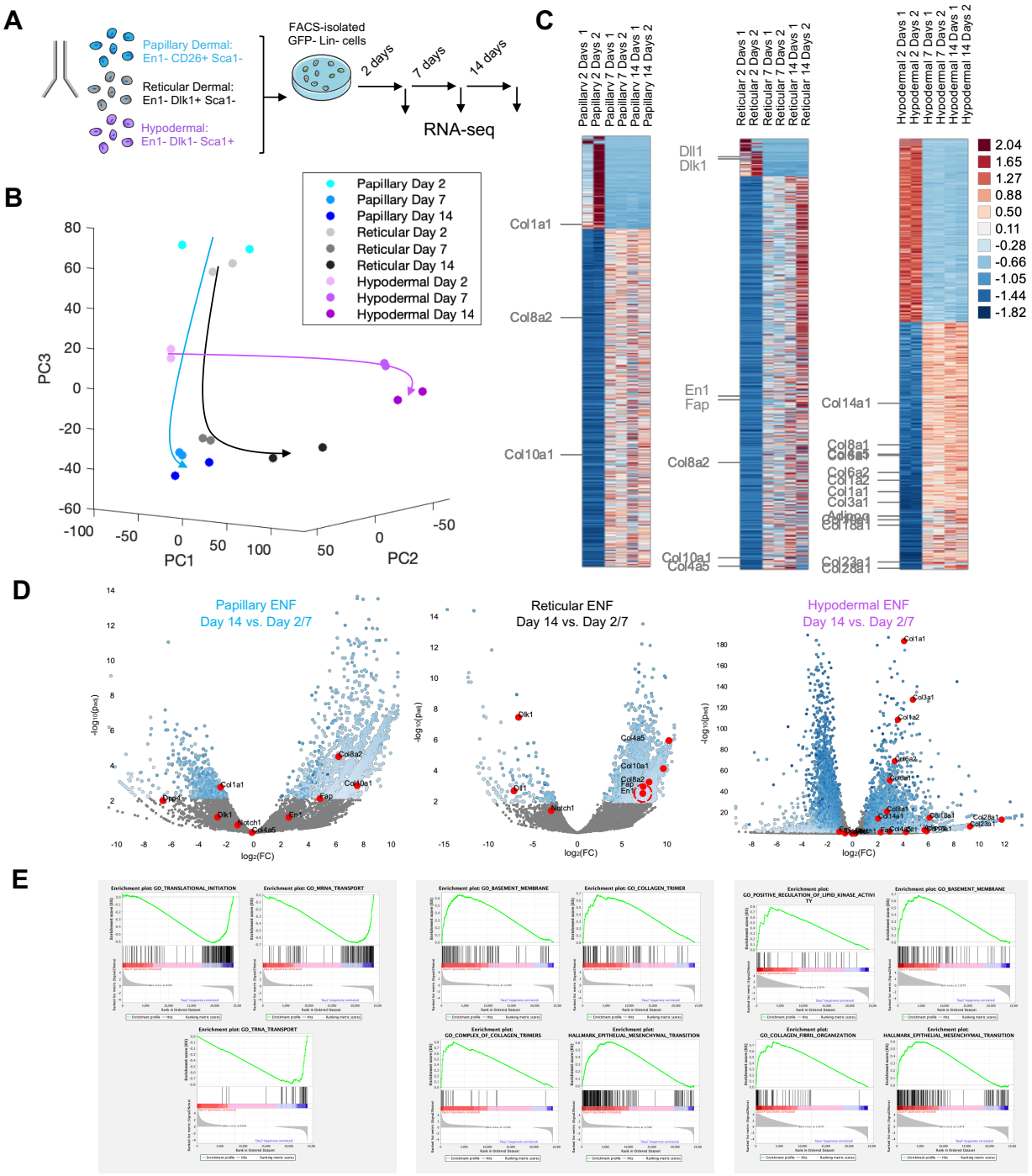


Figure S5: Bulk RNA-seq analysis of ENF subpopulations. (A) Schematic detailing FACS-isolation and culture of papillary (blue), reticular (gray), and hypodermal (purple) ENFs. **(B)** Principal components analysis of ENF subpopulations cultured on TCPS for 2, 7, and 14 days. **(C, D)** Gene expression heatmaps (C) and volcano plots (D) for papillary (left), reticular (middle), and hypodermal (right) ENFs. Specific genes that were significantly up- or down-regulated are labeled. Of note, only reticular ENFs showed upregulation of *En-1* in response to mechanical activation (red circle in volcano plot). **(E)** Gene set enrichment analysis of differentially expressed genes for

papillary (left), reticular (middle), and hypodermal (right) ENFs cultured on TCPS (Day 14 vs. Day2/7).

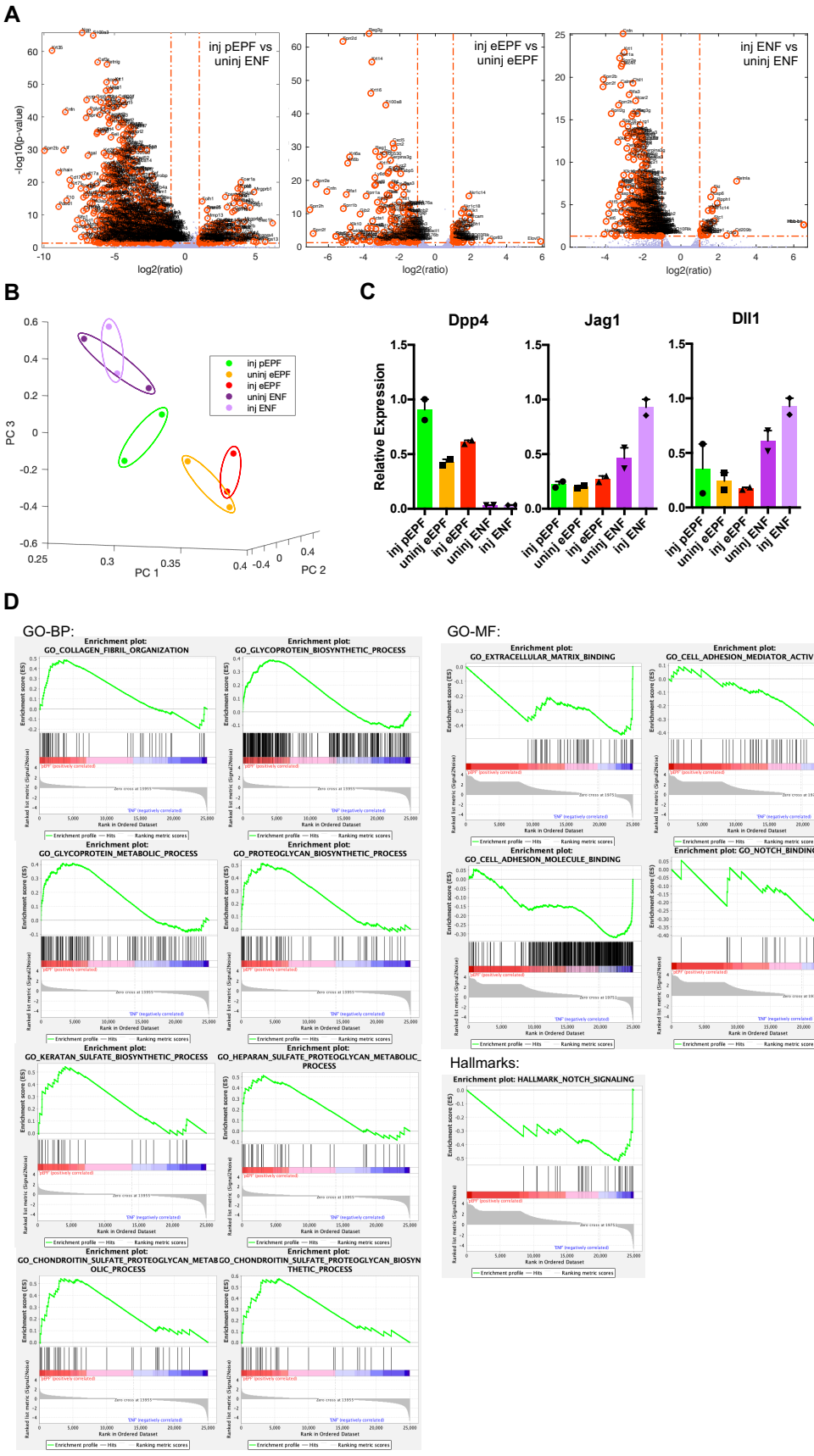

A
inj pEPF vs uninj ENF
inj eEPF vs uninj eEPF
inj ENF vs uninj ENF
-log10(p-value)
log2(ratio)
B
inj pEPF
uninj eEPF
inj eEPF
uninj ENF
inj ENF
PC 1
PC 2
PC 3
C
Dpp4
Jag1
Dll1
Relative Expression
D
GO-BP:
Enrichment plot: GO_COLLAGEN_FIBRIL_ORGANIZATION
Enrichment plot: GO_GLYCOPROTEIN_BIOSYNTHETIC_PROCESS
Enrichment plot: GO_GLYCOPROTEIN_METABOLIC_PROCESS
Enrichment plot: GO_PROTEOGLYCAN_BIOSYNTHETIC_PROCESS
Enrichment plot: GO_KERATAN_SULFATE_BIOSYNTHETIC_PROCESS
Enrichment plot: GO_HEPARAN_SULFATE_PROTEOGLYCAN_METABOLIC_PROCESS
Enrichment plot: GO_CHONDROITIN_SULFATE_PROTEOGLYCAN_METABOLIC_PROCESS
Enrichment plot: GO_CHONDROITIN_SULFATE_PROTEOGLYCAN_BIOSYNTHETIC_PROCESS
GO-MF:
Enrichment plot: GO_EXTRACELLULAR_MATRIX_BINDING
Enrichment plot: GO_CELL_ADHESION_MEDIATOR_ACTIVITY
Enrichment plot: GO_CELL_ADHESION_MOLECULE_BINDING
Enrichment plot: GO_NOTCH_BINDING
Hallmarks:
Enrichment plot: HALLMARK_NOTCH_SIGNALING

Figure S6: Gene set enrichment analysis for *in vivo* ENFs and pEPFs. (A) Volcano plot showing 1,138 genes significantly upregulated or downregulated in ENFs, eEPFs, or pEPFs in wounds (inj) compared to uninjured skin (uninj). Individual plots are labeled (top right corner) with comparisons shown in each plot. **(B)** PCA of RNA-seq data for pEPFs, eEPFs, and ENFs from injured and uninjured skin. **(C)** Comparison of Dpp4 (CD26; left panel), Jag1 (middle panel), and Dll1 (right panel) gene counts for each cell type. **(D)** Normalized RNA-seq counts for scar ENFs (GFP^- $CD26^-$) and postnatal EPFs (GFP^+) were analyzed for enrichment in the Gene Ontology Biological Process, Gene Ontology Molecular Function, and Hallmark databases. Scar ENFs were enriched for ECM-adhesion and Notch signaling-related terms, supporting their mechanosensitive phenotype. In contrast, postnatal EPFs were enriched for a variety of ECM-related terms, confirming that activation of *Engrailed-1* in the wound environment by mechanosensitive ENFs was associated with the acquisition of a pro-fibrotic phenotype.

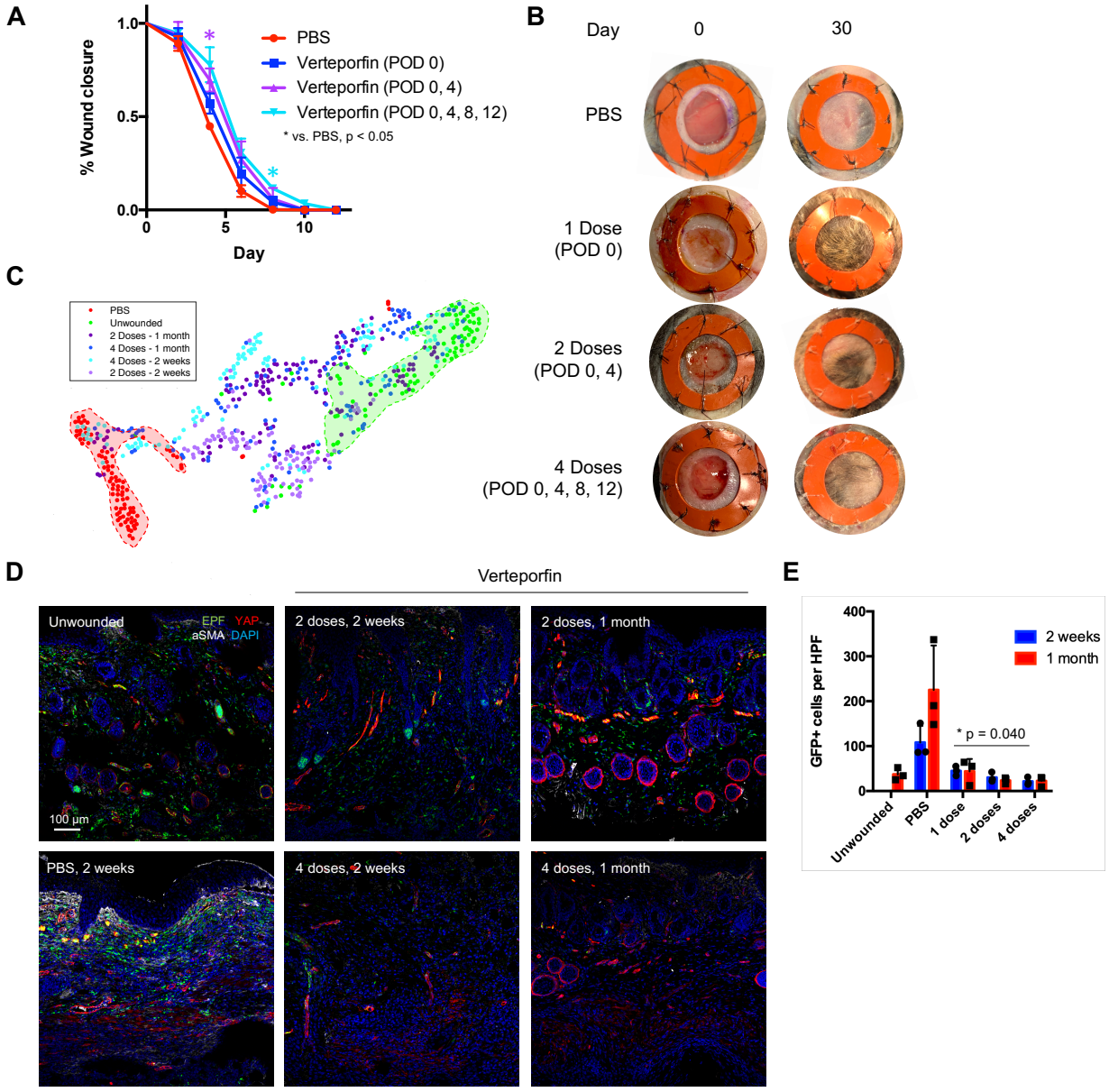


Figure S7: Characterization of wounds treated with multiple doses of Verteporfin. (A) Wound curve showing closure (re-epithelialization) rates for wounds treated with PBS (red) versus 1 (blue), 2 (purple), or 4 (light blue) doses of Verteporfin at indicated intervals. N = at least 6 wounds/condition. POD 4, 2 dose Verteporfin vs. PBS, **P* = 0.0140; POD 8, 4 dose Verteporfin vs. PBS, **P* = 0.0140; all other comparisons, *P* > 0.05. **(B)** Representative gross photographs of wounds treated with PBS (first row), 1 (second row), 2 (third row), or 4 (fourth row) doses of Verteporfin at POD 0 (left column) and 30 (right column). **(C)** t-SNE visualization of ECM ultrastructural properties for various treatment groups after 2 weeks or 1 month of healing (see legend). Clusters for unwounded skin and scar (PBS) highlighted by shaded regions. **(D)** Immunofluorescent histology for EPFs (GFP+), YAP+, and α-SMA+ cells in unwounded skin and PBS- or Verteporfin-treated scars. **(E)** Quantification of GFP+ cells (EPFs) per 20x HPF.

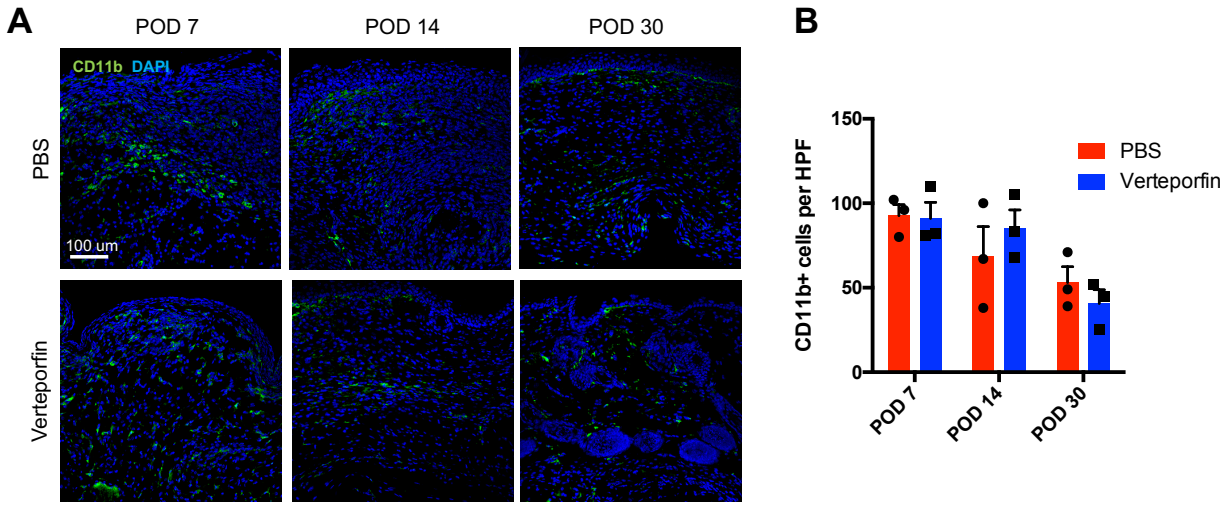
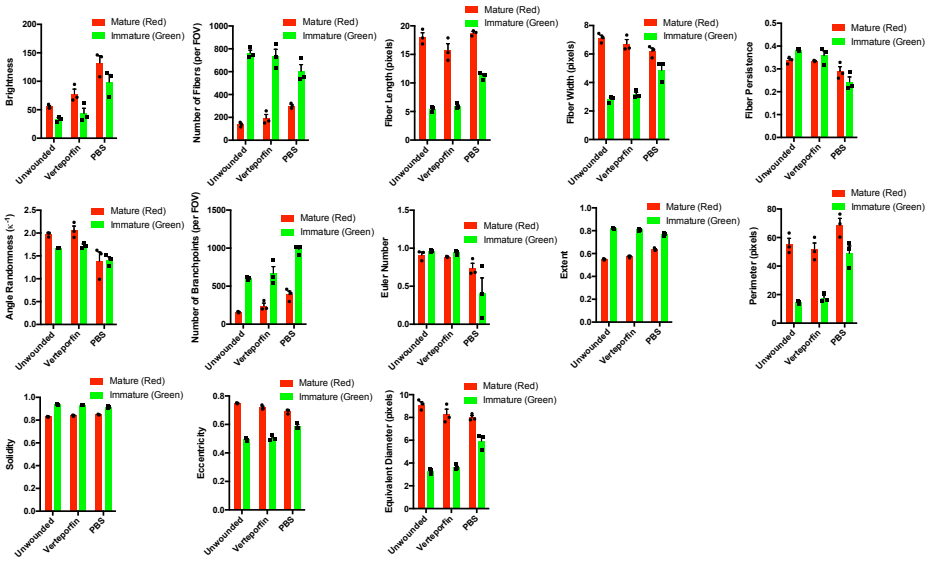


Figure S8: Comparison of immune cell infiltration (A) Immunofluorescent histology for CD11b+ myeloid cells (macrophages, neutrophils) in PBS- and Verteporfin-treated wounds at POD 7, 14, and 30. **(B)** Quantification of CD11b+ cells per 20x HPF (all comparisons n.s.).

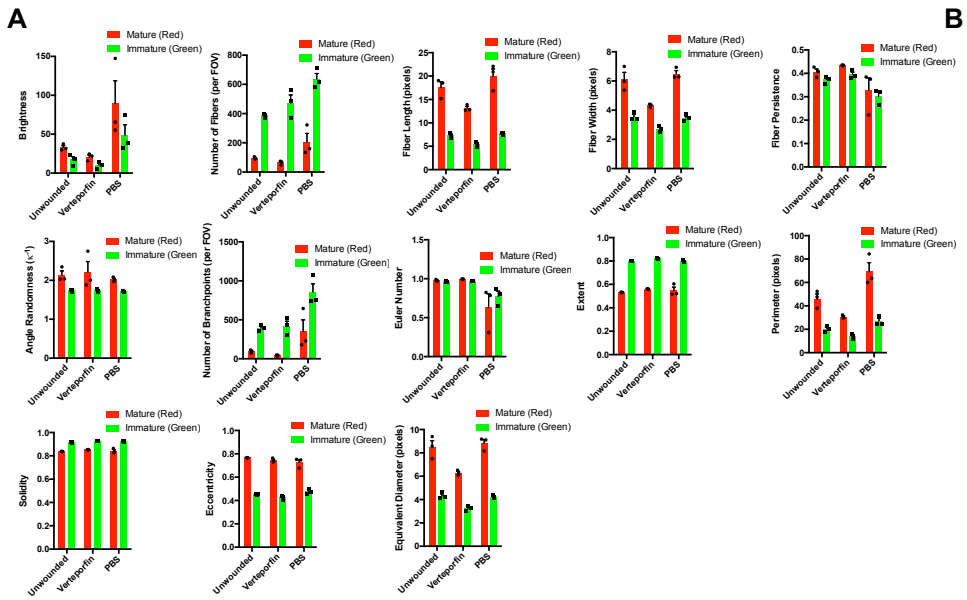
A



B

	Unwounded vs. PBS	Unwounded vs. Verteporfin
Brightness Red	0.02153639	0.1402572
Number of Fibers Red	0.00538111	0.3269987
Length Red	0.4129471	0.3414797
Width Red	0.1541445	0.4836084
Persistence Red	0.2225794	0.7726405
Angle Randomness Red	0.1120161	0.35783
Number of Branchpoints Red	0.04318765	0.1465099
Euler Number Red	0.2278618	0.614072
Extent Red	0.00702982	0.1134568
Perimeter Red	0.2432686	0.7009157
Solidity Red	0.01408811	0.1807911
Eccentricity Red	0.02441917	0.1122365
Equivalent Diameter Red	0.1147097	0.3550306
Brightness Green	0.05382544	0.4843854
Number of Fibers Green	0.04283562	0.6891531
Length Green	0.00057345	0.2041019
Width Green	0.02108803	0.1593481
Persistence Green	0.02048721	0.3818789
Angle Randomness Green	0.06779243	0.2906806
Number of Branchpoints Green	0.01352199	0.5897527
Euler Number Green	0.1128322	0.5234231
Extent Green	0.0207322	0.1770478
Perimeter Green	0.01688782	0.2103601
Solidity Green	0.05457558	0.4012164
Eccentricity Green	0.02372114	0.51771
Equivalent Diameter Green	0.00971158	0.1554854

Figure S9: Quantification of ECM fiber parameters at 2 weeks following wounding. (A) Quantified fiber parameters from unwounded skin and Verteporfin- or PBS-treated wounds at POD 14. Separate values were calculated for mature (red) versus immature (green) fibers, as assessed by Picrosirius staining. Dots represent the average of two wounds from each of N = 3 mice. **(B)** P-values for comparison of fiber parameters (red, mature; green, immature) between unwounded skin and either PBS- (left) or Verteporfin-treated wounds (right).

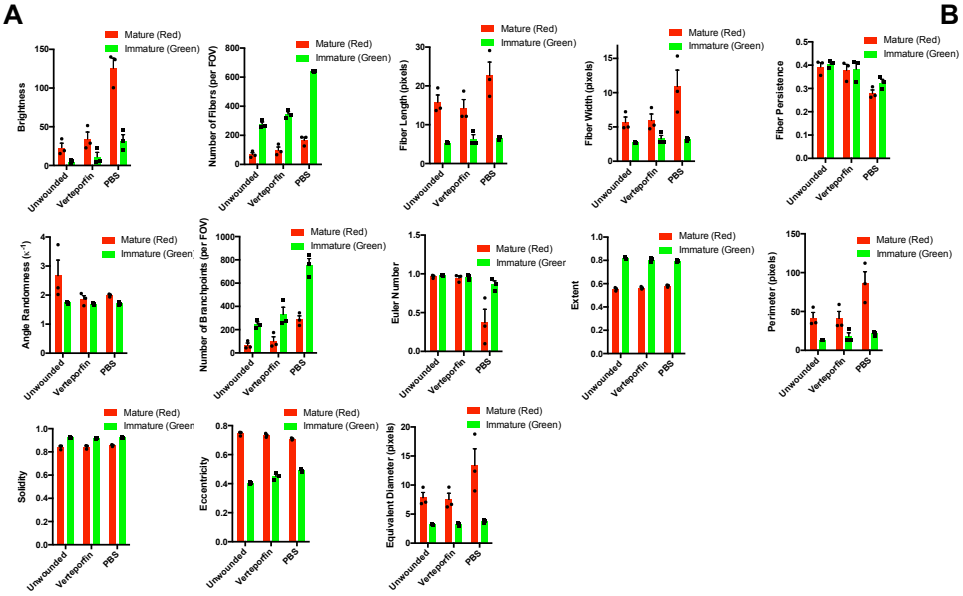


B

	Unwounded vs. PBS	Unwounded vs. Verteporfin
Brightness Red	0.2097743	0.1312494
Number of Fibers Red	0.2126086	0.1320496
Length Red	0.02739428	0.05412324
Width Red	0.6187718	0.04964219
Persistence Red	0.2938966	0.1565951
Angle Randomness Red	0.2462229	0.8373055
Number of Branchpoints Red	0.24889	0.07081044
Euler Number Red	0.1890137	0.1330719
Extent Red	0.6190985	0.00017416
Perimeter Red	0.1796518	0.06297936
Solidity Red	0.7166296	0.00987068
Eccentricity Red	0.2211311	0.1461072
Equivalent Diameter Red	0.3620837	0.04968594
Brightness Green	0.2032939	0.4274585
Number of Fibers Green	0.03180603	0.3044375
Length Green	0.3511433	0.08773357
Width Green	0.7743711	0.07409608
Persistence Green	0.1241506	0.3845503
Angle Randomness Green	0.1981639	0.8216563
Number of Branchpoints Green	0.06770968	0.792688
Euler Number Green	0.1103502	0.6292602
Extent Green	0.6286606	0.0065861
Perimeter Green	0.2029898	0.1276798
Solidity Green	0.1714856	0.02375743
Eccentricity Green	0.1821141	0.07204964
Equivalent Diameter Green	0.7296221	0.07308456

Figure S10: Quantification of ECM fiber parameters at 1 month following wounding. (A) Quantified fiber parameters from unwounded skin and Verteporfin- or PBS-treated wounds at POD 30. Separate values were calculated for mature (red) versus immature (green) fibers, as assessed by Picrosirius staining. Dots represent the average of two wounds from each of N = 3 mice. **(B)** P-values for comparison of fiber parameters (red, mature; green, immature) between unwounded skin and either PBS- (left) or Verteporfin-treated wounds (right).

A



B

	Unwounded vs. PBS	Unwounded vs. Verteporfin
Brightness Red	0.03059	0.05980368
Number of Fibers Red	0.04305336	0.07942578
Length Red	0.3088515	0.05563198
Width Red	0.2282299	0.4567412
Persistence Red	0.07291049	0.1516946
Angle Randomness Red	0.3273398	0.2193545
Number of Branchpoints Red	0.03098891	0.1719919
Euler Number Red	0.08240213	0.3410646
Extent Red	0.01261546	0.3145702
Perimeter Red	0.166312	0.750848
Solidity Red	0.3369204	0.2289237
Eccentricity Red	0.02094451	0.3966395
Equivalent Diameter Red	0.2664189	0.3438208
Brightness Green	0.09438984	0.3166877
Number of Fibers Green	0.00124827	0.0033322
Length Green	0.01458173	0.4694655
Width Green	0.0352634	0.3843226
Persistence Green	0.07831585	0.4097577
Angle Randomness Green	0.5246977	0.01334708
Number of Branchpoints Green	0.02068713	0.2092807
Euler Number Green	0.1477682	0.328719
Extent Green	0.04595456	0.3384725
Perimeter Green	0.03901946	0.4315653
Solidity Green	0.673828	0.3951294
Eccentricity Green	0.00592039	0.1310256
Equivalent Diameter Green	0.0281797	0.4035656

Figure S11: Quantification of ECM fiber parameters at 3 months following wounding. (A) Quantified fiber parameters from unwounded skin and Verteporfin- or PBS-treated wounds at POD 90. Separate values were calculated for mature (red) versus immature (green) fibers, as assessed by Picrosirius staining. Dots represent the average of two wounds from each of N = 3 mice. **(B)** P-values for comparison of fiber parameters (red, mature; green, immature) between unwounded skin and either PBS- (left) or Verteporfin-treated wounds (right).

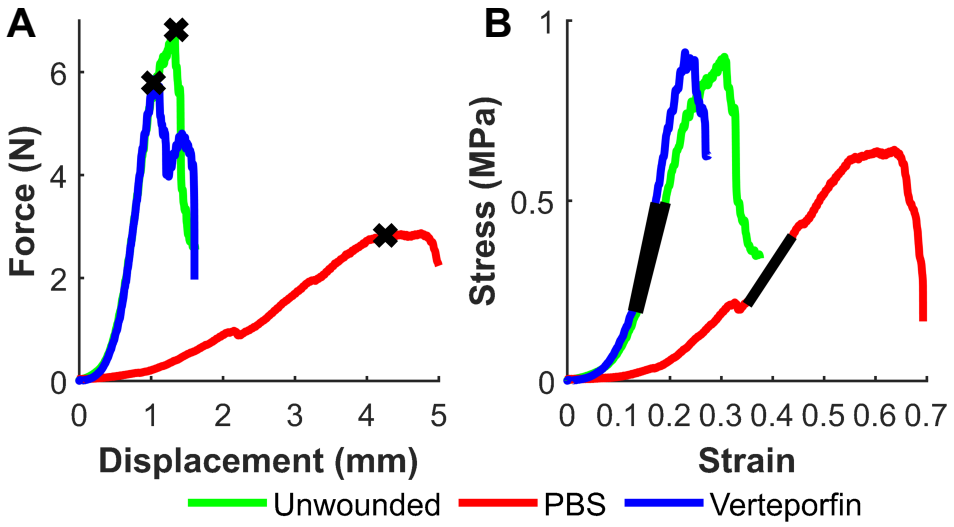


Figure S12: Instron comparison of PBS- and Verteporfin-treated wounds after 1 month of healing (A) Representative force-displacement curve for unwounded skin (green), PBS-treated wounds (red), and Verteporfin-treated wounds (blue) after 1 month of healing. **(B)** Representative stress-strain curve for the same groups as (A). Verteporfin treatment yielded wounds that more closely resembled unwounded skin than scar (PBS treatment) after 1 month of healing.

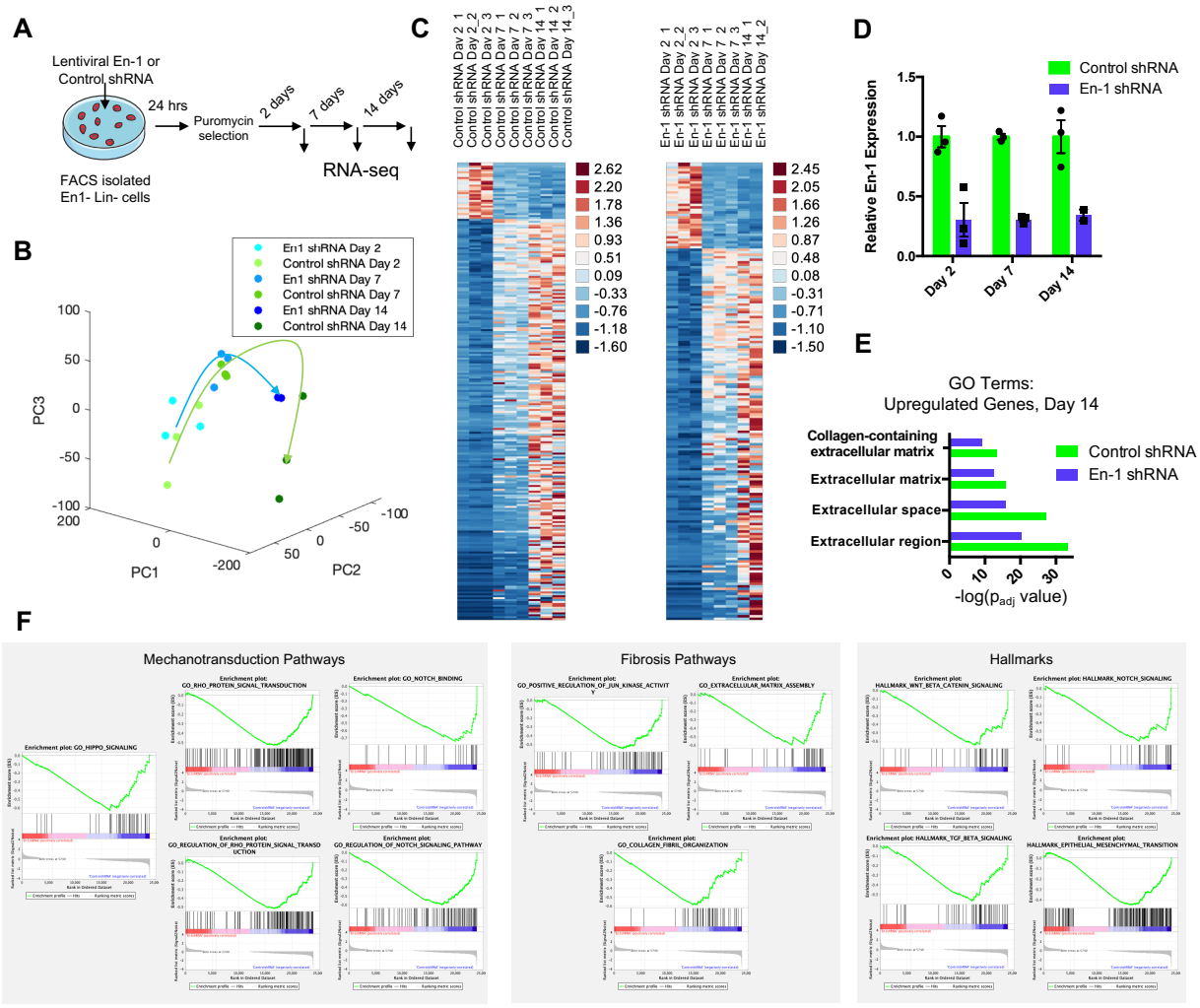


Figure S13: Bulk RNA-seq analysis of *Engrailed-1* shRNA knockdown. (A) Schematic detailing FACS-isolation, culture, and lentiviral shRNA-mediated knockdown of *En-1* expression in ENFs. **(B)** Principal components analysis of control- and *En-1*-shRNA treated ENFs cultured on TCPS for 2, 7, and 14 days. **(C)** Gene expression heatmaps for control- (left) and *En-1*-shRNA (right) ENFs. **(D)** Quantification of relative *En-1* transcription at Day 2, 7, and 14 of culture. N = 3 wells with pooled ENFs derived from separate litters. **(E)** GO term enrichments for significantly upregulated genes after 14 days of culture on TCPS. **(F)** Gene set enrichment analysis of differentially expressed genes in control- and *En*-1-shRNA treated ENFs after 14 days of culture.