

Supplemental Information

Targeted silencing of Engrailed-1 reprograms profibrotic fibroblast lineage for scarless wound healing

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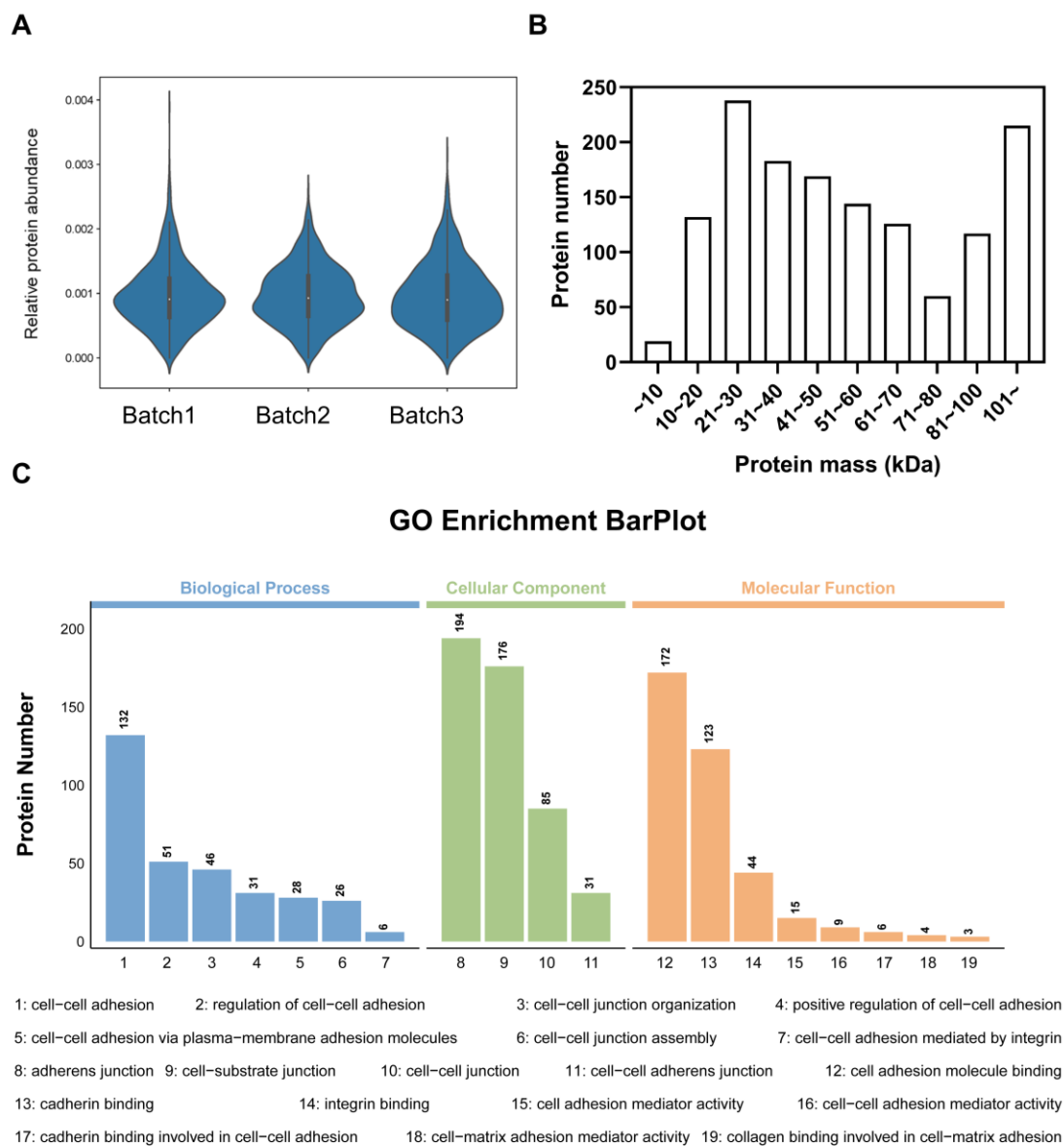


Figure. S1 (A) Relative protein abundance of membrane proteins on FibroMC. Batch1, Batch2, and Batch3 represent three batches of FibroMC samples. (B) Protein mass distribution of identified proteins. (C) GO enrichment analysis of functional proteins.

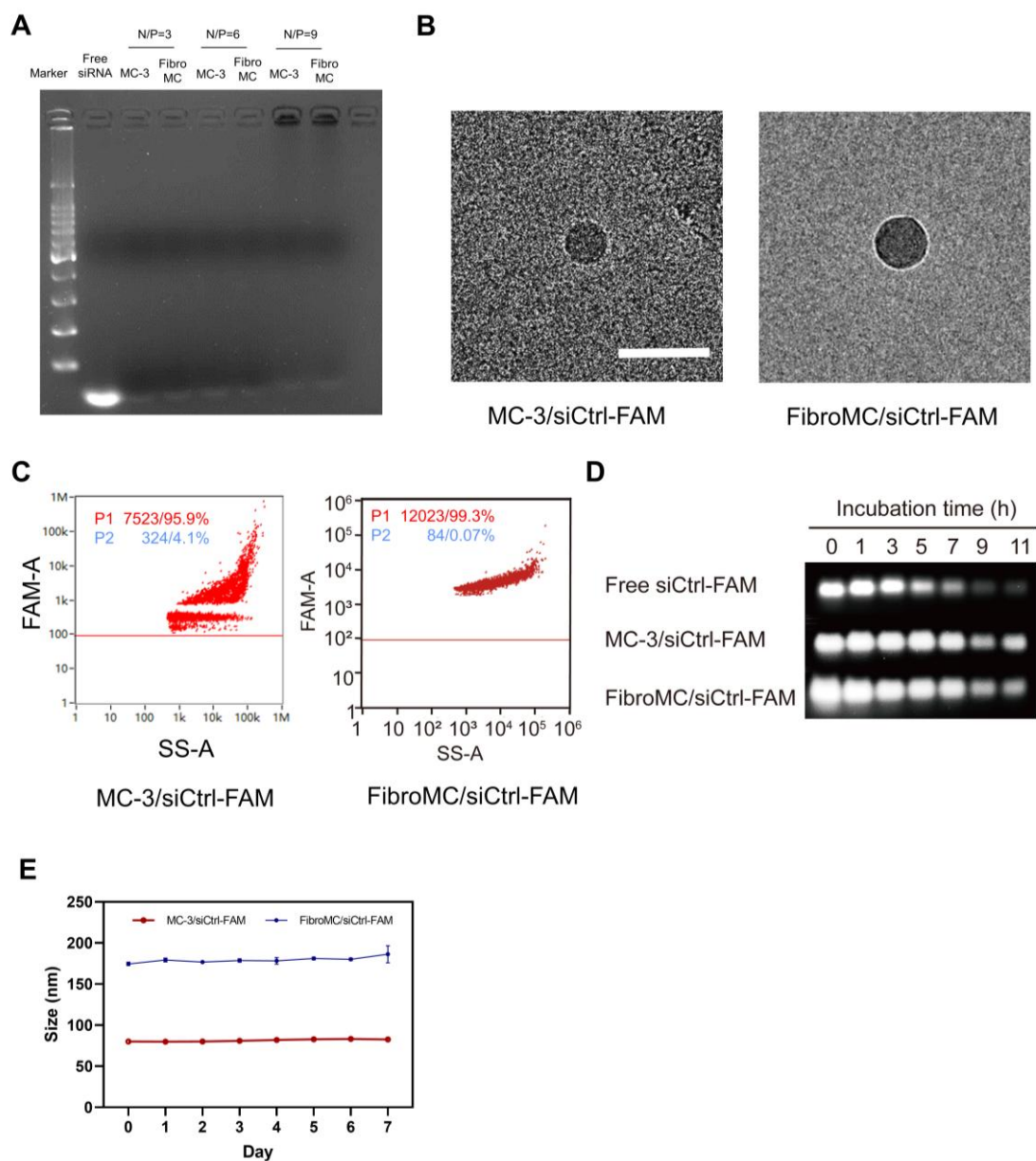


Figure. S2 (A) Gel retardation assays of siRNA/MC-3 or FibroMC with various N/P ratios. (B) Representative TEM images of MC-3/siCtrl-FAM and FibroMC/ siCtrl-FAM. Scale bar = 100 nm. (C) Encapsulation efficacy of MC-3and FibroMC detected by Flow NanoAnalyzer. (D) Electrophoresis strips of free siCtrl-FAM, MC-3/siCtrl-FAM and FibroMC/siCtrl-FAM after incubation with 50% FBS, 37°C. (E) Stability of MC-3/siCtrl-FAM and FibroMC/ siCtrl-FAM in PBS, 4°C. Data are present as the mean $\pm$ SD.

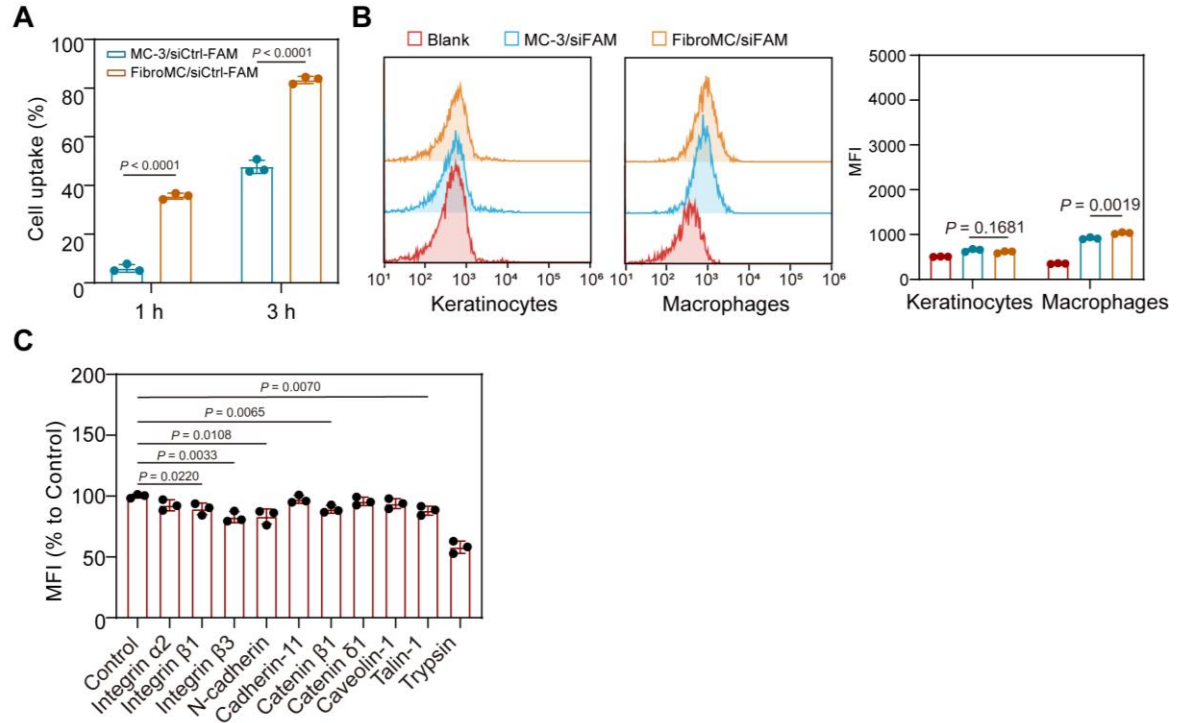


Figure. S3 (A) Quantification of cell uptake efficiency after 1-hour and 3-hour incubation with different groups. (B) Flow cytometry analysis of indicated nanoparticle labeled with FAM on keratinocytes and RAW264.7 cells, after 1-hour incubation. (C) Fibroblasts uptake of FibroMC/siCtrl-FAM in presence of blocking antibodies (anti-integrin $\alpha 2$, anti-integrin $\beta 1$, anti-integrin $\beta 3$, anti-N-cadherin, anti-Cadherin-11, anti-Catenin $\beta 1$, anti-Catenin $\delta 1$, anti-Caveolin-1, or anti-Talin-1 antibodies) or trypsin treatment of FibroMC/siCtrl-FAM (n = 3 per group). Data are present as the mean $\pm$ standard deviation (SD). Statistical analysis was calculated using the Student t-test (n $\geq$ 3).

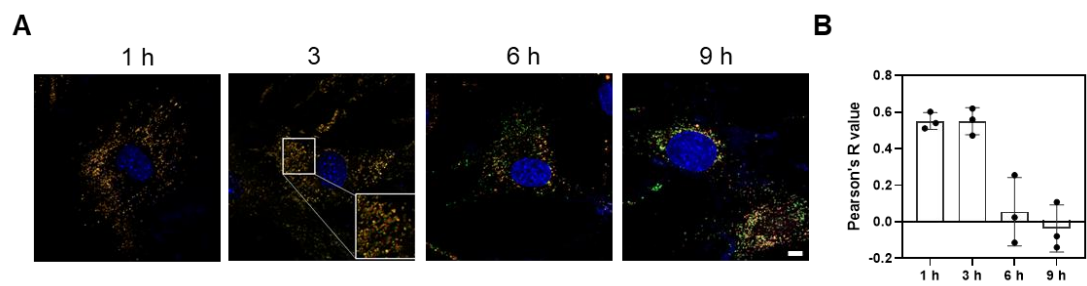


Figure. S4 (A) Biodistribution of MC-3/siCtrl-FAM (green) in fibroblasts in different intervals. Nucleus were stained with DAPI (blue), lysosomes were stained with lyso-tracker (red). Scale bar =10 μ m. (B) Pearson's correlation coefficient of the fluorescence colocalization between FAM and LysoTracker™ Red DND-99 in fibroblasts, as calculated using ImageJ software.

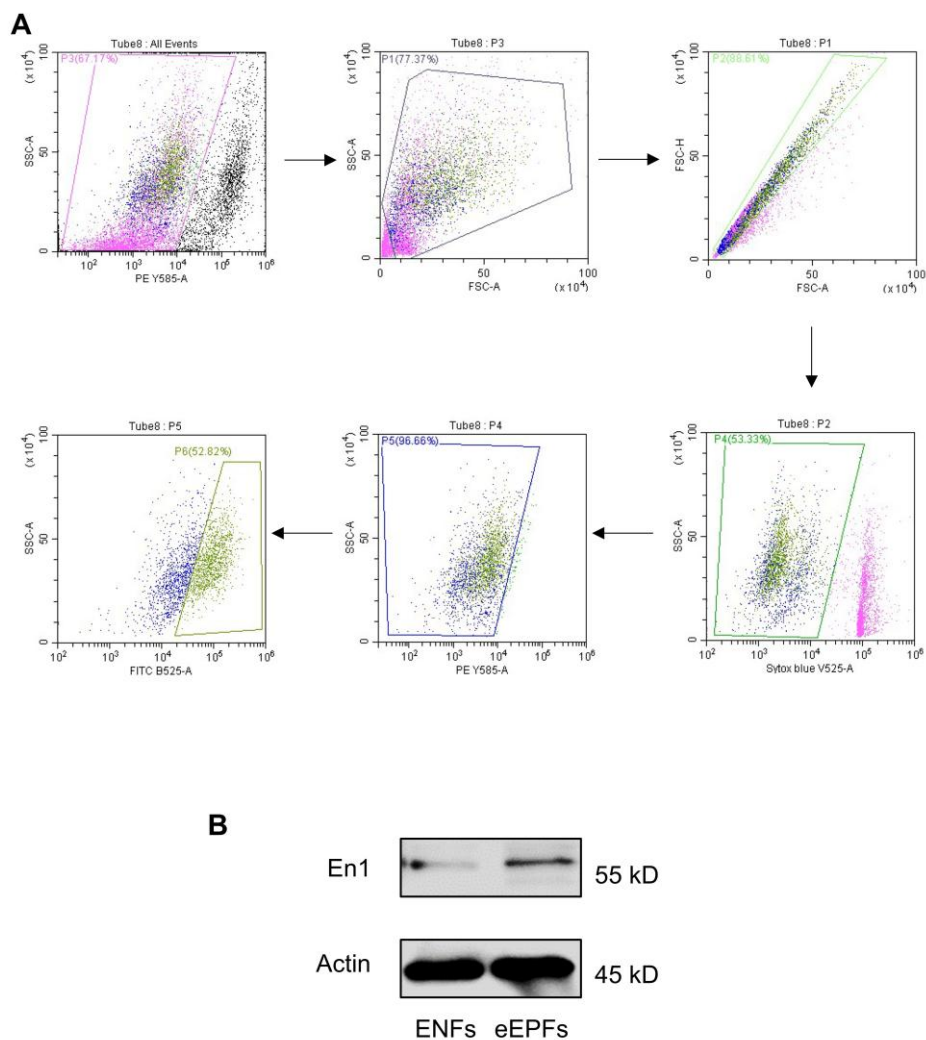


Figure. S5 (A) Flow sorting of eEPFs. (B) Representative Western blots of En1 expression in sorted ENFs and eEPFs.

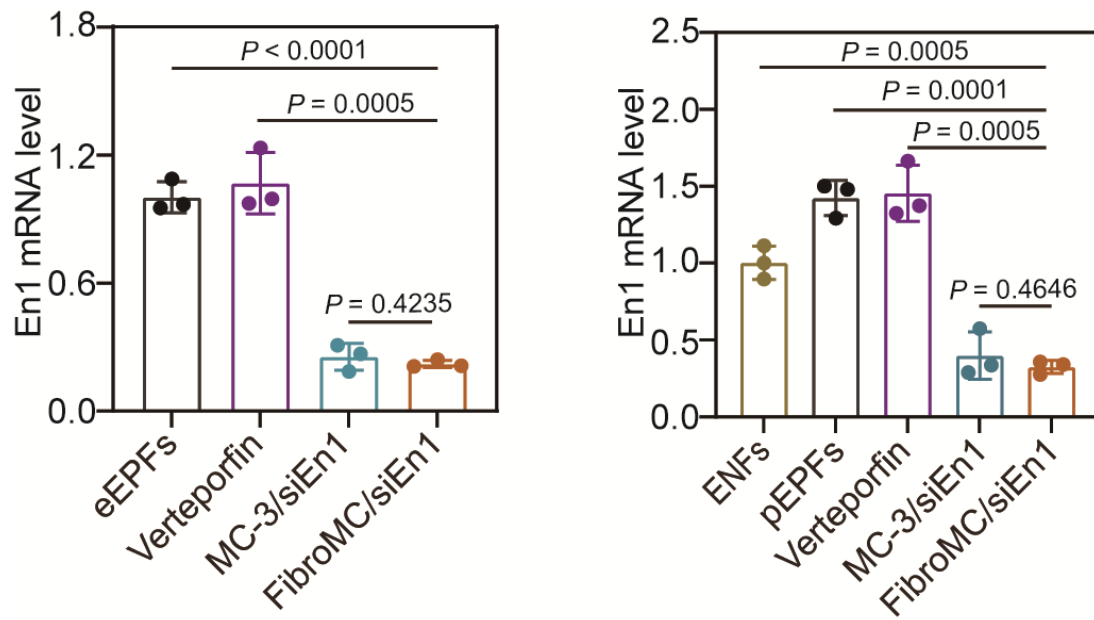


Figure. S6 Relative En1 mRNA levels in eEPFs and pEPFs post treatment after 48 h with three formulations (verteporfin, MC-3/siEn1, and FibroMC/siEn1).

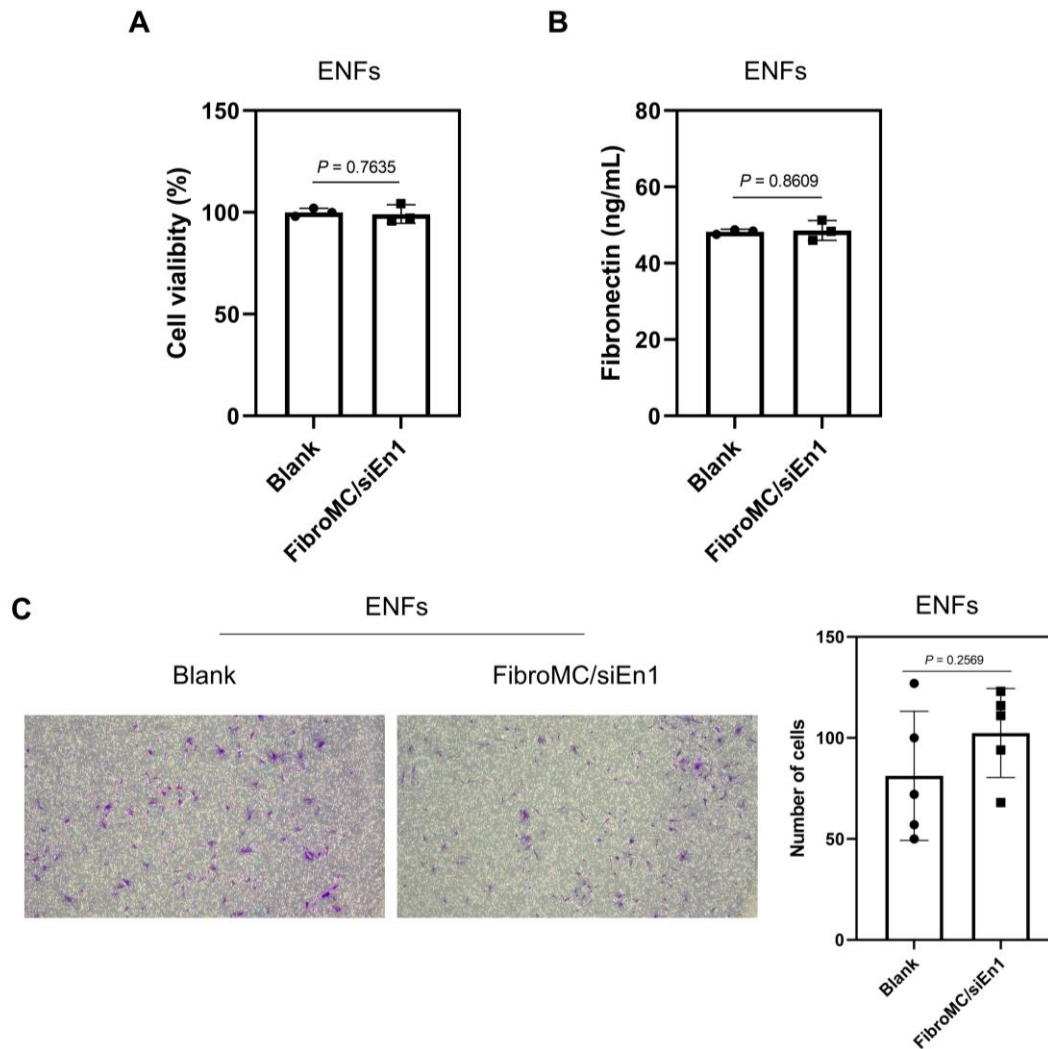


Figure. S7 (A) Viability of ENFs upon incubation with FibroMC/siEn1 for 48 h. (B) Relative fibronectin expression in ENFs upon incubation with FibroMC/siEn1 for 48 h, detected by ELISA kit. (C) Images and quantitative analysis of transwell assay results of ENFs upon incubation with FibroMC/siEn1 for 48 h.

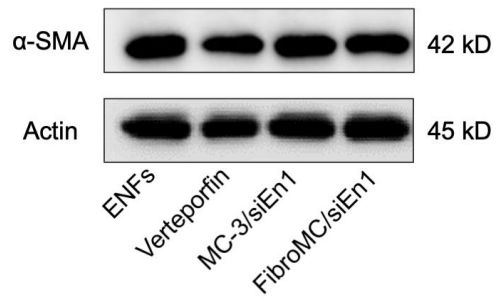


Figure. S8 Representative Western blots of α -SMA expression in ENFs treated with different groups.

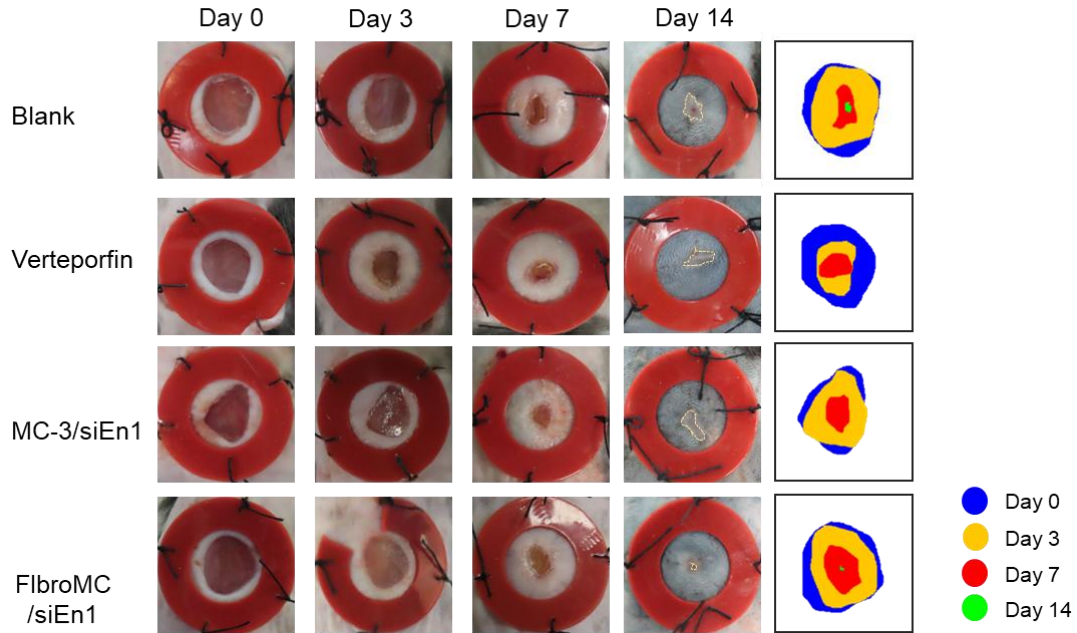
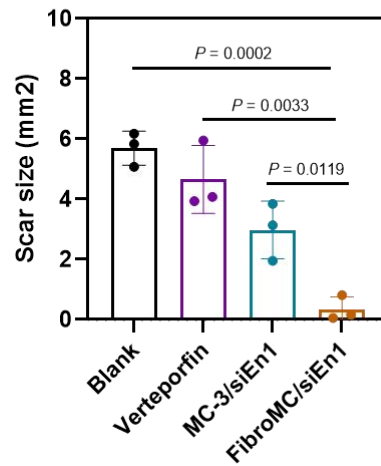
A**B**

Figure. S9 (A) Gloss appearance images of the wound sites across various groups on days 0, 3, 7, 14. (B) Quantitative evaluation and statistical analysis of scar size in Blank, Verteporfin, MC-3/siEn1 and FibroMC/siEn1 on Day 14. Data are presented as the mean $\pm$ standard deviation (SD). Statistical analysis was calculated using Student t-test ($n \geq 3$).

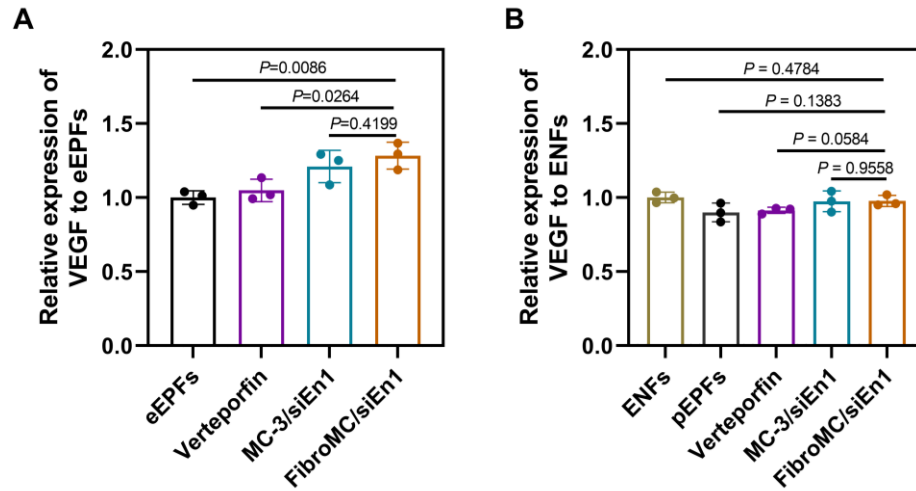


Figure. S10 (A) Relative VEGF expression in eEPFs treated with different group, detected by ELISA kit. (B) Relative VEGF expression in pEPFs treated with different group, detected by ELISA kit. Statistical analysis was calculated using the Student t-test ($n \geq 3$).

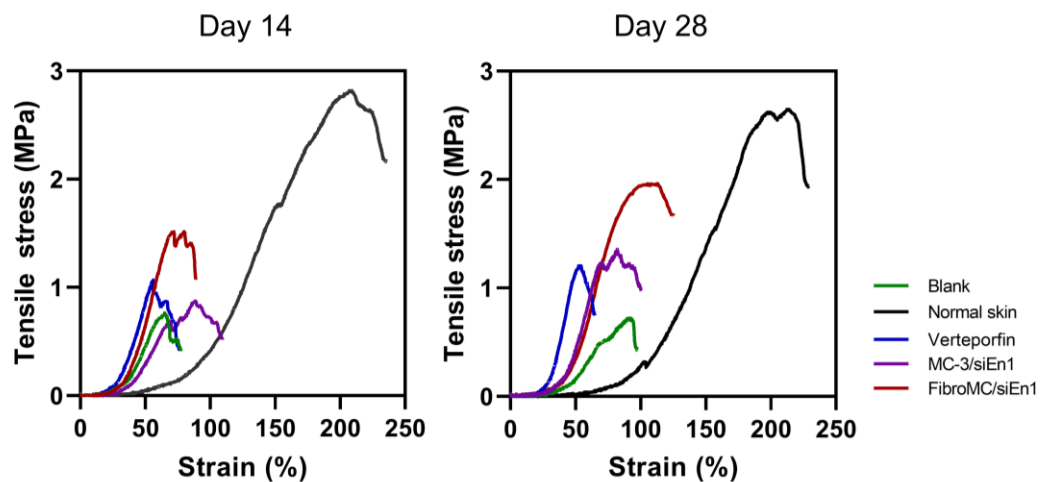


Figure. S11 Representative stress-strain curve for normal skin, Blank, Verteporfin, MC-3/siEn1 and FibroMC/siEn1 treated skin after 14 days and 28 days healing.

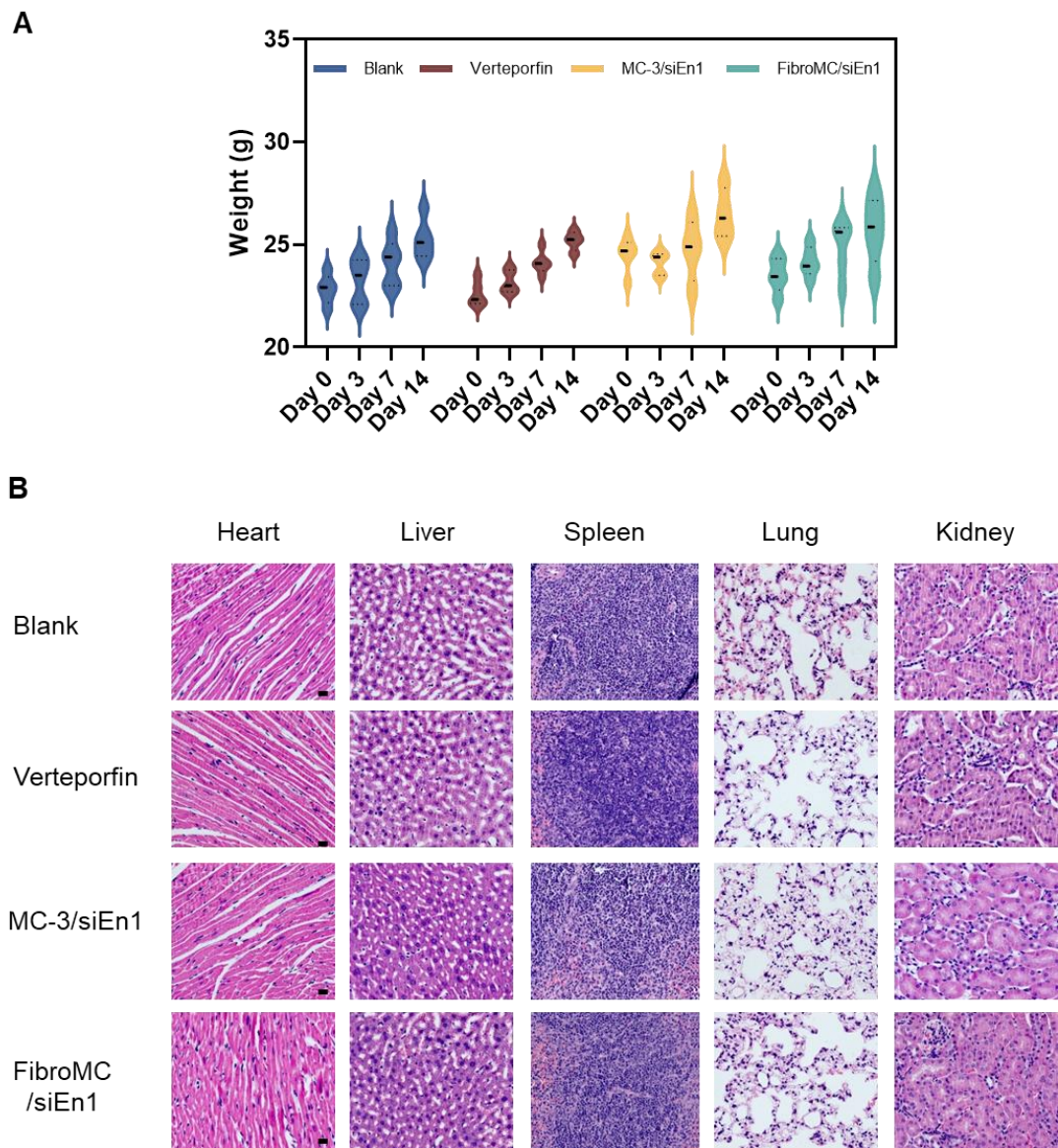


Figure. S12 (A) Body weight of different groups from Day 0 to Day 14. (B) Representative H&E staining images of heart, liver, spleen, lung and kidney on day 14. Scale bar, 20 μ m.

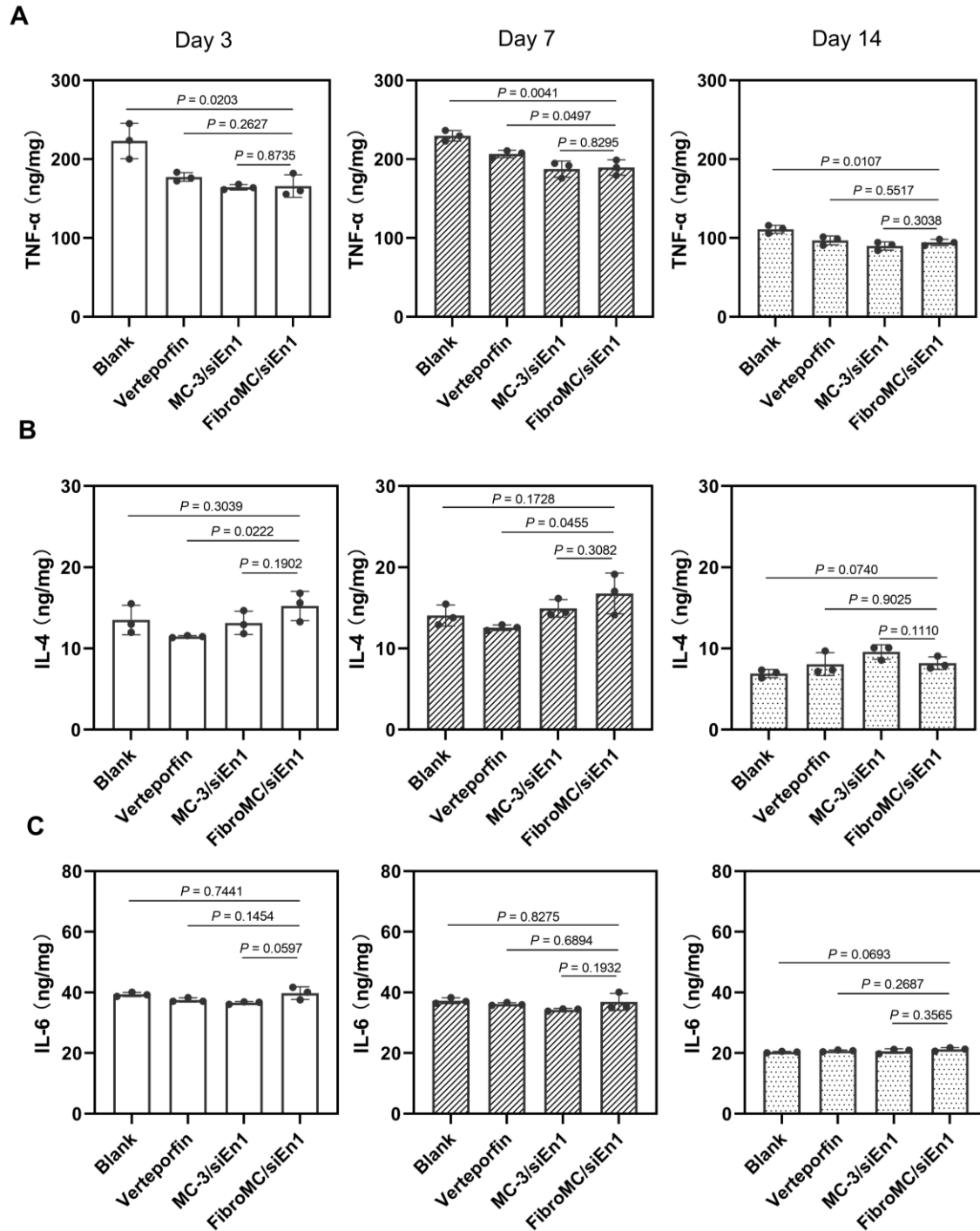


Figure. S13 (A) TNF- α expression in skins treated with different group in day 3,7,14, detected by ELISA kit. (B) IL-4 expression in skins treated with different group in day

3,7,14, detected by ELISA kit. (B) IL-6 expression in skins treated with different group
in day 3,7,14, detected by ELISA kit.

Table S1. Primers used for quantitative real-time PCR

Target gene	Forward primer sequence	Reverse primer sequence
<i>En1</i>	5'-TCCTACTCATGGGTTCCG-3'	5'-TGAGTCAGTCTTGACCAC-3'
<i>β-actin</i>	5'-TCTTTGATGTCACGCACGAT-3'	5'-TACAGCTTCACCACCACA-3'
<i>Col1a1</i>	5'-GAAGCACGTCTGGTTTGGA-3'	5'-ACTCGAACGGGAATCCATC-3'
<i>Col1a2</i>	5'-CCAACAAGCATGTCTGGTTAGGA- 3'	5'-TCAAACCTGGCTGCCACCAT -3'
<i>Acta2</i>	5'-ATGCCTCTGGACGTACAACG-3'	5'-CACACCATCTCCAGAGTCCA-3'