

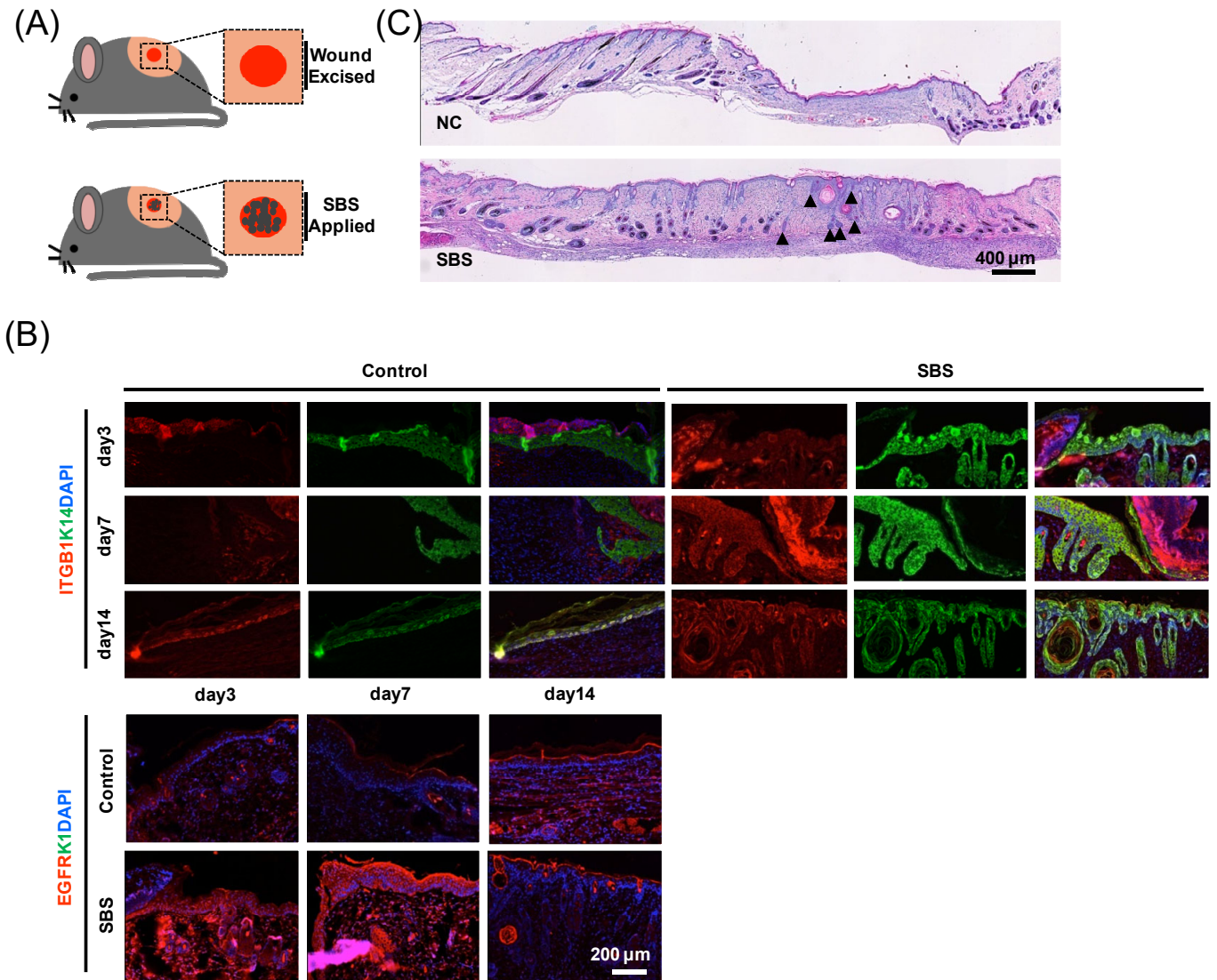
# **Comprehensive Proteomic Atlas of Skin Biomatrix Scaffolds Reveals a Supportive Microenvironment for Epidermal Development**

## **Supplementary Files**

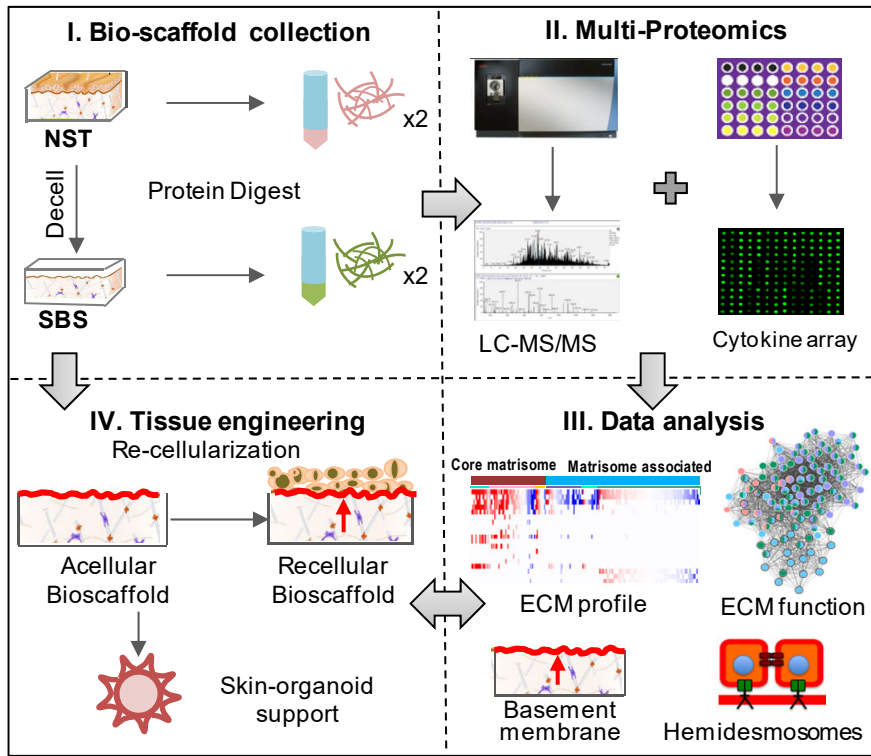
### **Abbreviations**

AGRN: Agrin; ASPN: Asporin; BM: basement membrane; BGN: Biglycan; CS: chondroitin sulfate; CLDN1: Claudin 1; DCN: Decorin; DSG1: Desmosomal Glycoprotein 1; DC: direct contact; EpSCs: epidermal stem cells; EGF: Epidermal Growth Factor; ECM: extracellular matrix; FGF: Fibroblast Growth Factor; FBN: fibrillin; GAG: glycosaminoglycan; HE: hematoxylin/eosin; HSPG: Heparan Sulfate Proteoglycan; HS: heparin sulfate; ITGA6: Integrin subunit alpha; ITGB4: Integrin Subunit Beta; IL: Interleukin; IFN: Interferon; CK14: Keratin 14; LBSs: liver biomatrix scaffolds; LUM: Lumican; MMP: matrix metalloproteinases; MBV: matrix-bound vesicles; NAFLD: nonalcoholic fatty liver disease; NID: nidogen; NST: native skin tissue; PLA2: phospholipase A2; PODN: Podocan; PG: proteoglycan; SBS: skin biomatrix scaffolds; SLRP: small leucine-rich proteoglycan; TGFB1: Transforming Growth Factor Beta 1; TNF: Tumor Necrosis Factor; VEGF: Vascular Endothelial Growth Factor; VCAN: Versican.

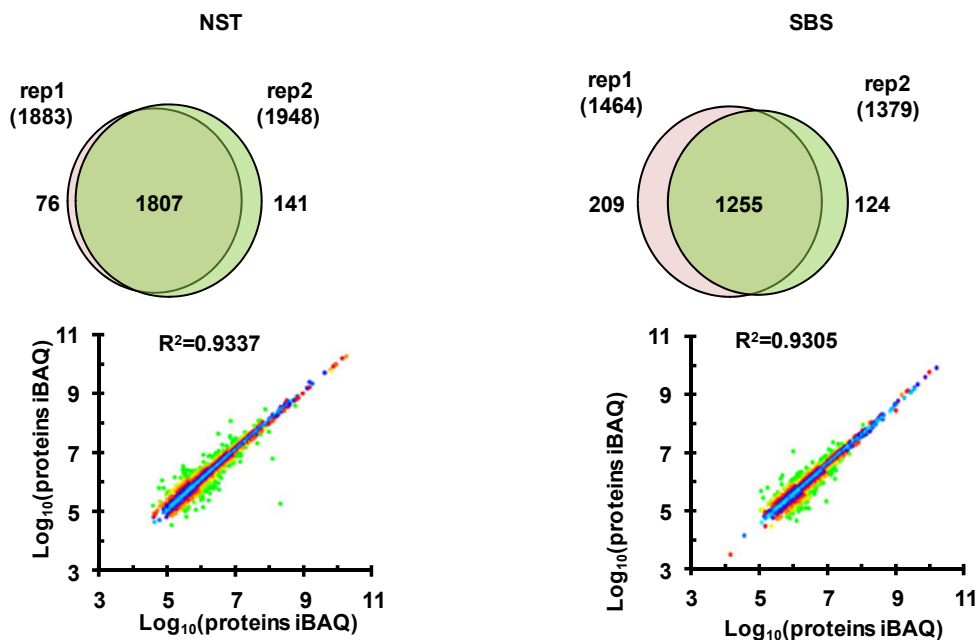
## Supplementary Figures



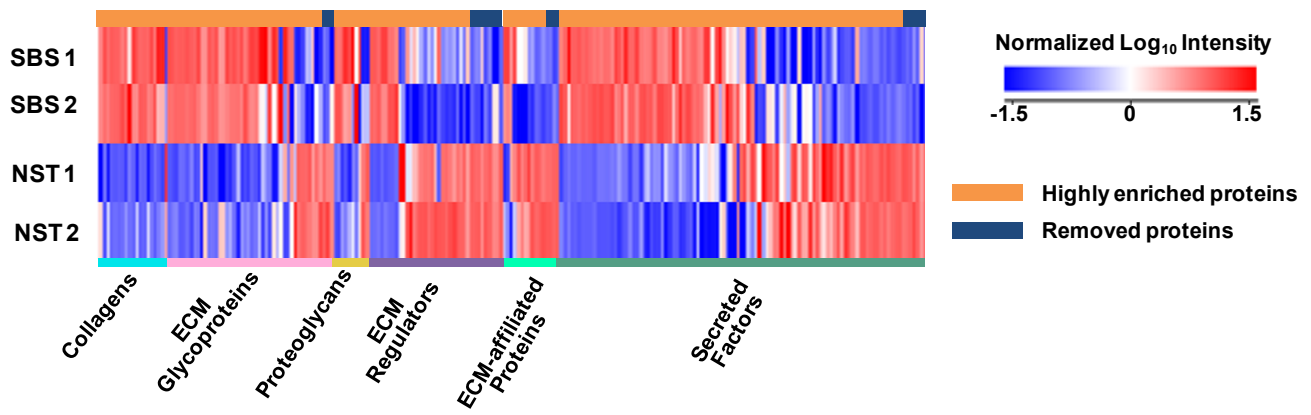
**Figure S1. Acellular skin biomatrix scaffolds support EpSCs growth during wound healing.** (A) A 6-mm diameter wound is excised from the back of the mouse (one on each side). (B) Hematoxylin/Eosin staining of mouse repaired skin on day 14 (scale bar: 400  $\mu\text{m}$ ). (C) Immunofluorescence analysis of ITGB1, K14, EGFR, and K1 during the process of mouse wound healing (days 3, 7, and 14) (scale bar: 200  $\mu\text{m}$ ).



**Figure S2. General workflow for decellularization, recellularization, and analyses strategies.**

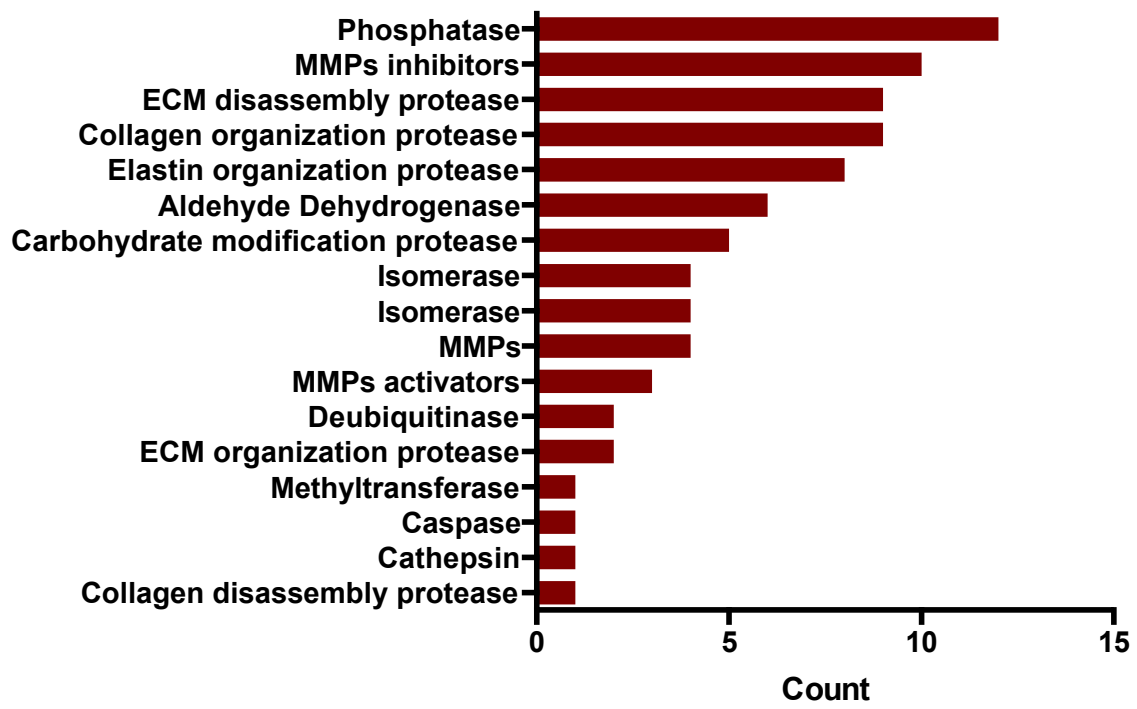


**Figure S3. Quality control of LC-MS/MS experiments.** Venn diagrams show the reproducibility of proteins from NST and SBS. There are 1,807 and 1,255 proteins commonly identified in two replicated proteomics experiments, respectively. Furthermore, the distributions of protein intensities of two repeat experiments of NST and SBS are correlated.



**Figure S4. Quantitative analysis of Matrisome proteins.** Heatmap based on normalized Log<sub>10</sub>Intensity of Matrisome proteins from skin biomatrix scaffolds (SBS) and normal skin tissue (NST). Six categories (cyan, pink, yellow, purple, green, and atrovirens lines) correspond to the six ECM components: collagens, glycoproteins, proteoglycans, regulators, ECM-affiliated proteins, and secreted factors. Orange and dark blue correspond to highly enriched proteins and proteins removed by decellularization (fold change >1/8 or <1/8, compared to NST). The red and blue boxes indicate proteins with increased and decreased abundance in SBS, respectively.





**Figure S6. Protease and protease inhibitor analysis in SBS.** The histogram shows the proteases and protease inhibitors in SBS. The X-axis represents the counts of different categories of proteases.

## Supplementary Tables

**Table S4. Functional categories and substrates of proteases/protease inhibitors in SBS**

| Categories                 | Biological process                                | Protease/<br>Protease Inhibitor  | Pro- or<br>Anti-<br>Effect | Substrates/<br>Functions                                     |
|----------------------------|---|--|----------------------------|--|
| Stem cell function         | Stem cell quiescence                              | HUWE1  | Pro-                       | N-Myc, Ascl1, Atoh1  |
|                            | Stem/progenitor cell mobilization and recruitment | MMP9   | Pro-                       | c-kit ligand<br>ECM  |
|                            | Cell Self-renewal                                 | CTSB, CUL4A, CUL3  | Pro-                       | Histone, SEC31   |
|                            |   | ALDH2, 3A2, 4A1, 7A1, 9A1, 18A1  | Anti-                      | RA synthesis   |
| Stem cell maintenance      | USP9X, USP7                                       | Pro-   | ubiquitin                  |  |
| Neovascularization         | Angiogenesis                                      | MMP1   | Pro-                       | PAR-1/NF- $\kappa$ B $\rightarrow$<br>VEGFR2, HSPG2,<br>VCAN |
|                            |   | MMP9   | Pro-                       | HSPG2  |
|                            |   | MMP13  | Pro-                       | HSPG2, VCAN  |
|                            |   | MMP1   | Pro-                       | ECM (collagen)   |
|                            |   | MMP1   | Anti-                      | unclear  |
|                            | Vascular remodeling                               | MMP3   | Pro-                       | ECM, pro-MMP-9   |
|                            |   | MMP9   | Pro-                       | N-cadherin, ECM,<br>VSMC-ECM<br>attachment                   |
| ECM remodeling             | Collagen organization                             | P4HA1, 2, PCOLCE, TNXB,<br>SERPINH1, PLOD1, 3, LOX,<br>COLGALT1  | Pro-                       | Collagen   |
|                            |   | A2M  | Anti-                      | Collagen   |
|                            | Elastin organization                              | MFAP2, 5, AMBP, LOX, FBN2,<br>EMILIN1, 2, SERPINB2   | Pro-                       | Elastin  |
|                            | ECM organization                                  | SPARC, VWA1  | Pro-                       | ECM  |
|                            |   | FBN1, 2, NID2, ASPN, FAP,<br>KLK7, PCYOX1, NPEPPS  | Anti-                      | ECM  |
|                            |   | PLG, MRC2, LGMN  | Pro-                       | MMPs   |
|                            | MMPs activators and inhibitors                    | TIMP1, 2, 4, ECM1,<br>SERPINC1, SERPING1, AMBP,<br>ZPZ, PCOLCE, SERPINB2   | Anti-                      | MMPs   |
|                            | Proteins and peptides                             | PPID, PPIA, FKBP, PDIA1,<br>PRMT1, CASP6, ACP1,<br>PPP1R7, PPP2CB, PPP2R1A,<br>2A, 5A, 5C, PPP3CA, PTPA,<br>PTPN1, 11, PTPRA | Pro-                       | Post-translational<br>Modification                           |
| Carbohydrate modifications | ST2B1, COLGALT1, UGT1A6,<br>RPN1, 2               | Pro-   | Carbohydrate,<br>collagen  |  |
| Others                     | Cell motility                                     | MMP9   | Pro-                       | TGF $\beta$  |
|                            | Tissue homeostasis                                | MMP9, 13   | Pro-                       | LAP  |



**Table S5. Companies that provided equipment, reagents and/or supplies**

| <b>REAGENT or RESOURCE</b>                  | <b>SOURCE</b>             | <b>IDENTIFIER</b> |
|---|---------------------------|-------------------|
| <b>Antibodies</b>                           |                           |                   |
| Alexa Fluor® 488 Goat anti-mouse IgG (H+L)  | Invitrogen                | Cat#A32723        |
| Alexa Fluor® 488 Goat anti-mouse IgG2a      | Invitrogen                | Cat#A-21134       |
| Alexa Fluor® 488 Goat Anti-Rabbit IgG (H+L) | Invitrogen                | Cat#A11008        |
| Alexa Fluor® 568 Goat anti-mouse IgG1       | Invitrogen                | Cat#A-21124       |
| Alexa Fluor® 568 Goat anti-mouse IgG2a      | Invitrogen                | Cat#A-21134       |
| Alexa Fluor® 568 Goat Anti-Rabbit IgG (H+L) | Invitrogen                | Cat#A11011        |
| Alexa Fluor® 647 Goat anti-mouse IgG2b      | Invitrogen                | Cat#A-21242       |
| Anti-rabbit IgG,HRP-linked Antibody         | Cell Signaling Technology | Cat#7074          |
| CLDN1                                       | Abcam                     | Cat#ab211737      |
| COL17A1                                     | Abcam                     | Cat#ab184996      |
| COL7A1                                      | Santa Cruz biotechnology  | Cat#sc-33710      |
| COL3A1                                      | Santa Cruz Biotechnology  | Cat#sc-271249     |
| Cytokeratin 1                               | Abcam                     | Cat#ab93652       |
| Cytokeratin 10                              | Abcam                     | Cat# ab9026       |
| Cytokeratin 14                              | Abcam                     | Cat#ab181595      |
| Cytokeratin 5                               | Abcam                     | Cat# ab17130      |
| DSG1  | Proteintech               | Cat#24587-1-AP    |
| EGFR  | Santa Cruz Biotechnology  | Cat#sc-377229     |
| GAPDH                                       | Proteintech               | Cat#HRP-6004      |
| Histone                                     | GeneTex                   | Cat#GTX122148     |
| IgG   | Bioss                     | Cat# bs-0293P-HRP |
| Integrinβ1                                  | Abcam                     | Cat#ab78502       |
| Integrinβ4                                  | Proteintech               | Cat#21738-1-AP    |
| Integrinα6                                  | Abcam                     | Cat#ab20142       |
| Involucrin                                  | Abcam                     | Cat#ab68          |
| m-IgGκ BP-HRP                               | Santa Cruz Biotechnology  | Cat#sc-516102     |
| Laminin                                     | Abcam                     | Cat#ab11575       |
| p63   | Abcam                     | Cat#ab735         |
| Ki-67                                       | Abcam                     | Cat#ab15580       |
| p-ERK 1/2                                   | Santa Cruz Biotechnology  | Cat#sc-136521     |
| p-GSK3β                                     | Cell Signaling Technology | Cat#5558S         |
| Plectin                                     | Proteintech               | Cat#sc-33649      |
| <b>Chemicals</b>                            |                           |                   |
| 0.25%Trypsin/EDTA-solution                  | Gibco                     | Cat##25200072     |
| advanced DMED/F-12 medium                   | Invitrogen                | Cat#12634         |
| B-27  | Gibco                     | Cat#17504         |
| complete protease inhibitors (PI)           | Roche                     | Cat#04693116001   |
| Dispase II                                  | Gibco                     | Cat#17150041      |

|   |                             |               |
|---|-----------------------------|---------------|
| EDGS  | Gibco                       | Cat#S0125     |
| EpLife medium   | Gibco                       | Cat#MEPI500CA |
| Fetal bovine serum  | Gibco                       | Cat#10099141  |
| Fluoro-Gel  | Electron Microscopy Science | Cat#17985-10  |
| Glutamax  | Gibco                       | Cat#35050     |
| hematoxylin   | Vector                      | Cat#H3404     |
| ITS-G   | Gibco                       | Cat#41400     |
| Matrigel  | Corning                     | Cat#354230    |
| penicillin/streptomycin                                     | Gibco                       | #15140163     |
| phospholipase A2 (PLA2)                                     | Sigma                       | Cat#P6534     |
| Protein A/G PLUS agarose                                    | Santa                       | Cat# sc-2003  |
| Protein Extraction Reagent Type 4                           | Sigma                       | Cat#C0356     |
| Sircol-Biocolour (soluble collagen assay)                   | Sircol-Biocolour            | Cat#S1000     |
| Blyscan assay (Blyscan, Sulfated glycosaminoglycan assay)   | Sircol-Biocolour            | Cat#B1000     |
| Fluorescent DNA Quantitation Kit                            | Bio-Rad Laboratories        | Cat#1702480   |
| Sodium Deoxycholate (SDC)                                   | Sigma                       | Cat#V900388   |
| Tributyl phosphine (TBP)                                    | Sigma                       | Cat# T7567    |
| NEAA  | Gibco                       | Cat#11140     |
| HEPES   | Gibco                       | Cat#15630     |
| N-Ace   | Sigma                       | Cat#A9165     |
| DTT   | Amresco                     | Cat#028       |
| IAA   | Sigma                       | Cat#11149     |
| AlbuMAX™ II Lipid-Rich BSA                                  | Gibco                       | Cat#11021     |
| Cytokines and small molecules                               |                             |               |
| rhEGF   | R&D                         | Cat#236-EG    |
| rhWnt3a   | R&D                         | Cat #5036-WN  |
| A83-01  | Sigma                       | Cat#SML0788   |
| Forsklin  | Selleck                     | Cat#S2449     |
| Critical Commercial Assays                                  |                             |               |
| ABC-HRP Kit   | Vectastain                  | Cat#PK-6200   |
| VECTOR® NovaRED™ Peroxidase (HRP) Substrate Kit             | Vector                      | Cat#SK-4800   |
| Avidin/Biotin Blocking Kit                                  | Vector                      | Cat#SP-2001   |
| Millipore Immobilon™ Western Chemiluminescent HRP Substrate | Millipore                   | Cat#WBKLS0100 |
| Pierce™ ECL Western Blotting Substrate                      | Thermo                      | Cat#32106     |
| <b>TISSUE RESOURCE</b>                                      | <b>SOURCE</b>               |               |
| Human foreskin tissues                                      | PLA 307 Hospital            |               |
| Fetal skin tissue   | Air Force General Hospital  |               |

|   |   |
|---|---|
| Experimental Models: Organisms/Strains            |   |
| Full-thickness skin from 3-month-old male piglets | SiPeiFu Biotechnology Company   |
| <b>SOFTWARE and ALGORITHMS</b>                    |   |
| Cytoscape   | version 3.6.1   |
| DAVID   | <a href="https://david.ncifcrf.gov/home.jsp">https://david.ncifcrf.gov/home.jsp</a>                 |
| iProX   | <a href="https://www.iprox.org/">https://www.iprox.org/</a>   |
| InForm  | PerkinElmer   |
| KEGG  | <a href="https://www.kegg.jp/kegg/pathway.html">https://www.kegg.jp/kegg/pathway.html</a>           |
| Matrisome Project                                 | <a href="http://matrisomeproject.mit.edu/">http://matrisomeproject.mit.edu/</a>                     |
| MaxQuant software                                 | version 1.5.8.3   |
| STRING database                                   | <a href="https://string-db.org/">https://string-db.org/</a>   |
| ProteomeXchange Consortium                        | <a href="http://proteomecentral.proteomexchange.org">http://proteomecentral.proteomexchange.org</a> |
| Perseus   | version 1.5.8.5   |
| UniProt   | <a href="https://www.uniprot.org/">https://www.uniprot.org/</a>                                     |
| <b>INSTRUMENT</b>                                 | <b>MANUFACTOR</b>   |
| Confocal microscope                               | Carl Zeiss Microscopy, Jena, Germany  |
| H7650 TEM   | Hitach, Tokyo, Japan  |
| Orbitrap Q-Exactive Mass Spectrometer             | Thermo Scientific™, Massachusetts, America  |
| Ultrasonic Cell Disruptor (Scientz-IID)           | Ningbo Scientz Biotechnology, Zhejiang, China   |
| Vectra  | PerkinElmer, Shelton, America   |
| Electric Dermatome                                | Xingmao, Shanxi, China  |
| ChemiDoc™ XRS+ with image Lab™ Software           | Bio-Rad, California, America  |
| Freezer Mill                                      | Spex SamplePrep, New Jersey, America  |