## Supplementary data

# Inducing hair follicle neogenesis with secreted proteins enriched in embryonic skin

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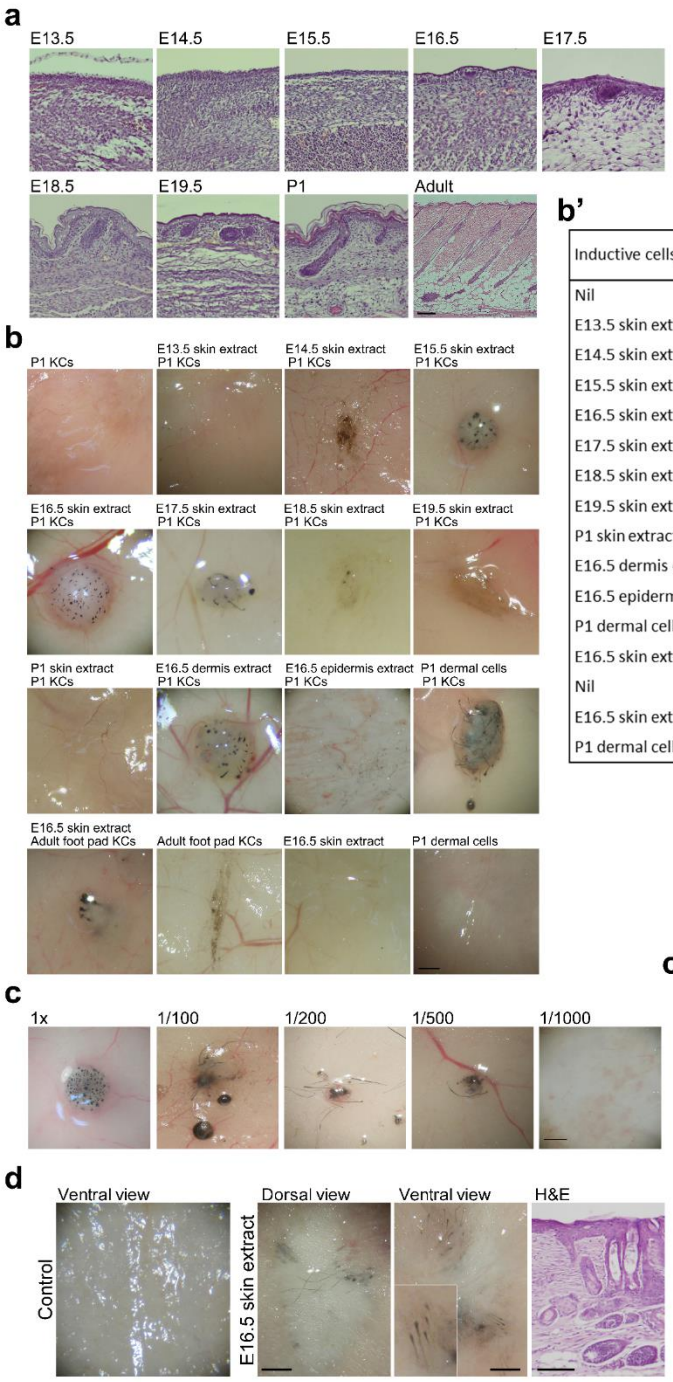



b’

| Inductive cells/ protein | KCs | Rate of hair follicle neogenesis % (n/n) |
|---|---|---|
| Nil | P1 KCs | 0% (0/30) |
| E13.5 skin extract | P1 KCs | 0% (0/10) |
| E14.5 skin extract | P1 KCs | 0% (0/10) |
| E15.5 skin extract | P1 KCs | 40% (4/10) # |
| E16.5 skin extract | P1 KCs | 70% (7/10) # |
| E17.5 skin extract | P1 KCs | 50% (5/10) # |
| E18.5 skin extract | P1 KCs | 0% (0/10) |
| E19.5 skin extract | P1 KCs | 0% (0/10) |
| P1 skin extract | P1 KCs | 0% (0/10) |
| E16.5 dermis extract | P1 KCs | 30% (3/10) # |
| E16.5 epidermis extract | P1 KCs | 0% (0/10) |
| P1 dermal cells | P1 KCs | 100% (10/10) # |
| E16.5 skin extract | Adult foot pad KCs | 20% (2/10) + |
| Nil | Adult foot pad KCs | 0% (0/10) |
| E16.5 skin extract | Nil | 0% (0/10) |
| P1 dermal cells | Nil | 0% (0/10) |

c’

| Concentration of E16.5 extract | KCs | Rate of hair follicle neogenesis % (n/n) |
|---|---|---|
| 1x | P1 KCS | 50.0% (5/10) |
| 1/100 | P1 KCS | 50.0% (5/10) |
| 1/200 | P1 KCS | 40.0% (4/10) |
| 1/500 | P1 KCS | 20.0% (2/10) |
| 1/1000 | P1 KCS | 0.0% (0/10) |

**Figure S1. The effect of cell-free whole-skin extract prepared from different developmental stages on HF neogenesis. (a)** Histology of Wistar rat skin at different developmental stages. In Wistar rats, embryonic HF development is delayed by ~1–1.5 days compared with mice. At E15.5, early hair placodes can be observed. Formation of hair germs with dermal condensates proceeds on E17.5. HFs develop to the short hair peg stage on E18.5. **(b-b’)** Effect of cell-free whole-skin extract on HF neogenesis. (**b**) Representative results of patch assays. (**b’)** Summary of patch assay results. Cell-free extracts from E15.5–17.5 whole skin induced new HFs from P1 KCs. For E16.5 skin,

dermal extract, but not epidermal extract, was able to induce HF neogenesis from P1 KCs, indicating that factors present in the dermis were sufficient to induce new HFs. E16.5 skin extract also induced HF neogenesis from adult KCs derived from hairless footpad skin (+ $p = 0.0767$). # $p < 0.05$, compared with P1 KCs only. **(c-c')** The effect of serial dilution of E16.5 skin extract on HF inductivity. (**c)** Representative results of patch assays. (**c')** Summary of patch assay results. E16.5 skin extract was serially diluted from 1 μg/μl (1×) to $1 \times 10^{-3}$ μg/μl (1/1000). Upon serial dilution, E16.5 skin extract gradually lost its ability to induce HFs, which disappeared completely by the 1/1000 dilution. **(d)** Effect of E16.5 extract on full-thickness wounds. In full-thickness wounds of nude mice, locally administered E16.5 extract induced HF neogenesis from transplanted C57BL/6 mouse keratinocytes (n=3). KC: keratinocyte. Bar: 100 μm in histology, 500 μm in gross images.

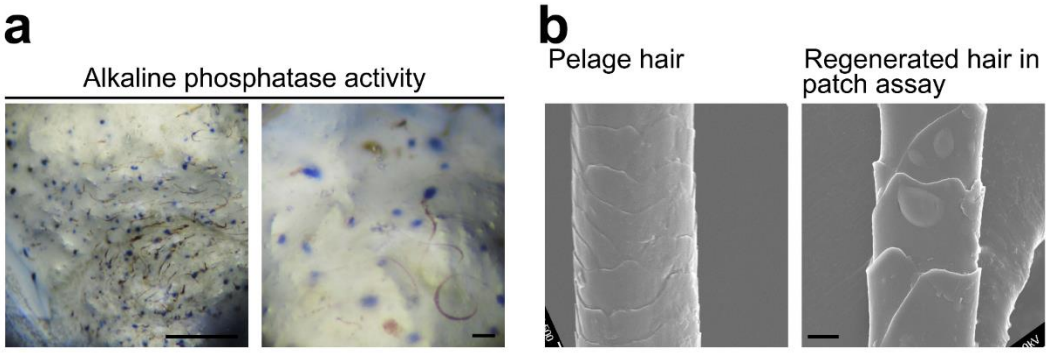



**Figure S2. Alkaline phosphatase activity and hair shaft morphology of new HFs induced by E16.5 skin extract. (a)** New HFs in patch assays exhibited alkaline phosphatase activity (blue color) in the proximal hair bulbs, marking new DPs. The right panel shows a magnified view. **(b)** Scanning electron micrographs of normal pelage hair and newly regenerated hair, both showing overlapping cuticles covering the hair shafts. Bar: 100 μm in (a), 10 μm in (b).

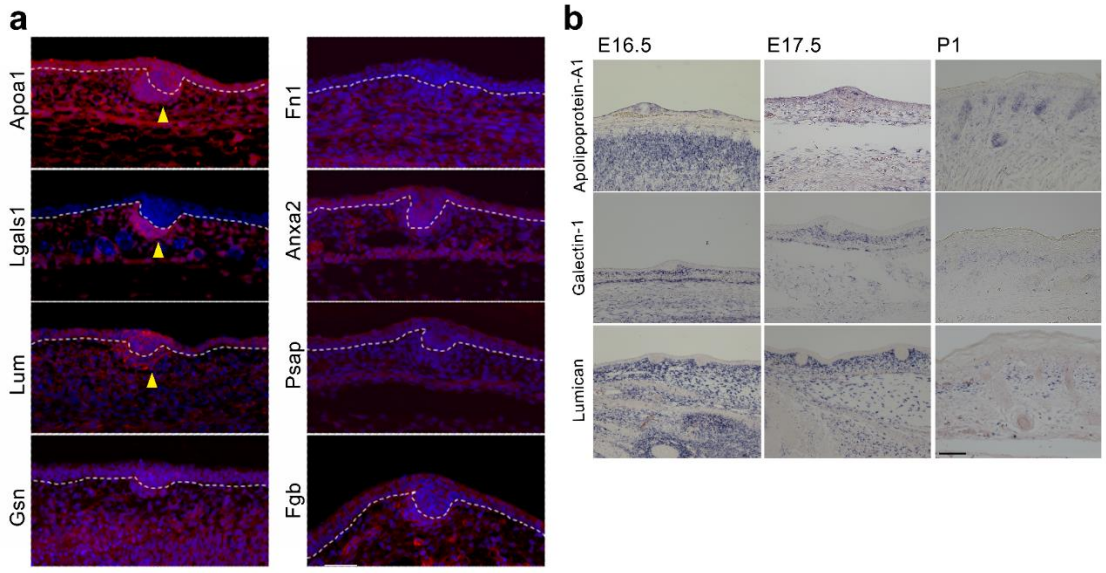
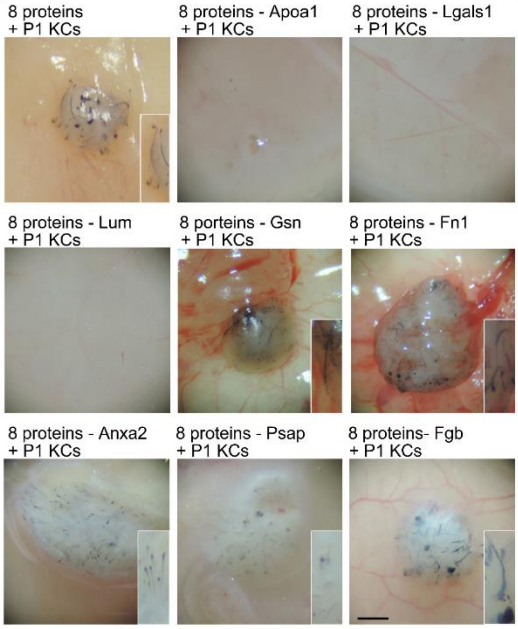



**Figure S3. Expression of specific proteins in rat embryonic skin. (a)** Immunostaining for the eight proteins in E17.5 Wistar rat embryonic skin. Compared with the seven other proteins, galectin-1 is preferentially localized to the dermis. Apoliporotein-A1, galectin-1 and lumican are also abundant in the dermal condensate (yellow arrowheads) of the developing HFs. Red: specific protein; blue: nuclear DAPI staining; dashed line: basement membrane. **(b)** *In situ* hybridization for the expression of apolipoprotein-A1, galectin-1 and lumican genes in the skin at different stages. All three genes are highly expressed in the mesenchyme of E16.5 and E17.5 embryonic skin. Expression of apolipoprotein-A1 in the epithelium was also observed in E16.5 and E17.5 skin. he expression level for all three genes in the dermis diminishes in P1 skin. Bar: 100 μm.

**a**




**b**

| Protein | KCs | Rate of hair follicle neogenesis % (n/n) |
|---|---|---|
| 8 proteins | P1 KCs | 60% (6/10) |
| 8 proteins – Apoa1 | P1 KCs | 0% (0/10) # |
| 8 proteins – Lgals1 | P1 KCs | 0% (0/10) # |
| 8 proteins - Lum | P1 KCs | 0% (0/10) # |
| 8 proteins – Gsn | P1 KCs | 50.0% (5/10) |
| 8 proteins – Fn1 | P1 KCs | 40.0% (4/10) |
| 8 proteins – Anxa2 | P1 KCs | 40.0% (5/10) |
| 8 proteins – Psap | P1 KCs | 40.0% (4/10) |
| 8 proteins – Fgb | P1 KCs | 40.0% (4/10) |

**Figure S4. Seven- or eight-protein combinations induce HF neogenesis. (a)** Representative patch assays. Bar: 500 μm. **(b)** Summary of patch assay results. Removal of Apoa1, Lgals1 or Lum from the eight-protein mixture ablated the ability to induce HF neogenesis. # $p < 0.05$, compared with the eight-protein mixture (n=10).

**Table S1**
Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of proteomes of E16.5 and P1 skin extract.

**Table S2**
RNA sequencing data of adult murine fibroblasts after exposure to E16.5 skin extract, Apoa1/Lgals1/Lum mix or each of the 3 proteins, murine E14.5 dermal fibroblasts and murine dermal papilla cells. Folds changes of gene expression in comparison with adult dermal fibroblasts were shown.

**Table S3**

Top 20 upregulated cellular processes in adult murine fibroblasts cultured in E16.5 extract, Apoa1/Lgals1/Lum mix, Apoa1, Lgals1, and Lum. Cellular processes shown in bold type indicate the ones shared with E16.5 extract treatment.

| **E16.5 extract** | **Apoa1/Lgals1/Lum mix** | **Apoa1** | **Lgals1** | **Lum** |
|---|---|---|---|---|
| Pathways in cancer | **Pathways in cancer** | **Pathways in cancer** | **Pathways in cancer** | **Pathways in cancer** |
| Focal adhesion | **MAPK signaling** | **MAPK signaling** | **MAPK signaling** | Cytokine-cytokine receptor interaction |
| Regulation of actin cytoskeleton | **Focal adhesion** | Cytokine-cytokine receptor interaction | Cytokine-cytokine receptor interaction | **MAPK signaling** |
| MAPK signaling | **Regulation of actin cytoskeleton** | Ribosome | Ribosome | **Focal adhesion** |
| Chemokine signaling | **Chemokine signaling** | **Regulation of actin cytoskeleton** | **Focal adhesion** | **Regulation of actin cytoskeleton** |
| Axon guidance | **Endocytosis** | **Focal adhesion** | **Regulation of actin cytoskeleton** | Ribosome |
| Endocytosis | Calcium signaling | **Chemokine signaling** | **Chemokine signaling** | **Chemokine signaling** |
| Ubiquitin mediated proteolysis | **Axon guidance** | Calcium signaling | Leukocyte transendothelial migration | Jak-STAT signaling |
| Wnt signaling | **Wnt signaling** | **Axon guidance** | **Neurotrophin signaling** | **Axon guidance** |
| Insulin signaling | **Tight junction** | Leukocyte transendothelial migration | **Axon guidance** | **Neurotrophin signaling** |
| TGF-beta signaling | **Neurotrophin signaling** | **Neurotrophin signaling** | **ECM-receptor interaction** | Leukocyte transendothelial migration |
| Neurotrophin signaling | **Insulin signaling** | B cell receptor signaling | T cell receptor signaling | **Lysosome** |
| Tight junction | Vascular smooth muscle contraction | **ECM-receptor interaction** | **Small cell lung cancer** | Toll-like receptor signaling |

| | | | | |
|---|---|---|---|---|
| Lysosome | Leukocyte transendothelial migration | T cell receptor signaling | Vascular smooth muscle contraction | Natural killer cell mediated cytotoxicity |
| Cell cycle | **Ubiquitin mediated proteolysis** | Vascular smooth muscle contraction | Oocyte meiosis | **ECM-receptor interaction** |
| Small cell lung cancer | **Cell cycle** | Natural killer cell mediated cytotoxicity | Fc gamma R-mediated phagocytosis | T cell receptor signaling |
| Prostate cancer | **Melanogenesis** | **Lysosome** | B cell receptor signaling pathway | Vascular smooth muscle contraction |
| ECM-receptor interaction | GnRH signaling pathway | **Small cell lung cancer** | Toll-like receptor signaling pathway | VEGF signaling |
| Melanogenesis | **Gap junction** | VEGF signaling | Fc epsilon RI signaling | B cell receptor signaling |
| Gap junction | ErbB signaling | Fc epsilon RI signaling | NOD-like receptor signaling | Hematopoietic cell lineage |