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LETTERS

Microenvironmental reprogramming of thymic epithelial cells to skin multipotent stem cells

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The thymus develops from the third pharyngeal pouch of the anterior gut and provides the necessary environment for thymopoiesis (the process by which thymocytes differentiate into mature T lymphocytes) and the establishment and maintenance of self-tolerance[1–3]. It contains thymic epithelial cells (TECs) that form a complex three-dimensional network organized in cortical and medullary compartments, the organization of which is notably different from simple or stratified epithelia[4]. TECs have an essential role in the generation of self-tolerant thymocytes through expression of the autoimmune regulator Aire[5,6], but the mechanisms involved in the specification and maintenance of TECs remain unclear[7–9]. Despite the different embryological origins of thymus and skin (endodermal and ectodermal, respectively), some cells of the thymic medulla express stratified-epithelium markers[10–12], interpreted as promiscuous gene expression. Here we show that the thymus of the rat contains a population of clonogenic TECs that can be extensively cultured while conserving the capacity to integrate in a thymic epithelial network and to express major histocompatibility complex class II (MHC II) molecules and Aire. These cells can irreversibly adopt the fate of hair follicle multipotent stem cells when exposed to an inductive skin microenvironment; this change in fate is correlated with robust changes in gene expression. Hence, microenvironmental cues are sufficient here to re-direct epithelial cell fate, allowing crossing of primitive germ layer boundaries and an increase in potency[13].

TECs were isolated from embryonic, post-natal or adult thymus obtained from wild-type or enhanced green fluorescent protein (EGFP) rats[14] and cultured in conditions used in human cell therapy[15]; under these conditions, TECs formed progressively growing colonies (refs 16, 17 and Y.B. and H. Green, unpublished data). Embryonic TECs were labelled with a fluorescent anti-EpCAM antibody and sorted. (EpCAM, epithelial cell adhesion molecule.) $EpCAM^-$ cells never formed colonies, whereas 0.1–0.5% of the $EpCAM^+$ cells did (Fig. 1a). Moreover, both $EpCAM^+UEA\text{-}1^+$ (medullary TEC) and $EpCAM^+UEA\text{-}1^-$ (cortical TEC) sorted cells gave rise to colonies (Fig. 1b), consistent with previous observations that separate progenitors exist for the cortical and medullary compartments[18,19]. (UEA-1, *Ulex Europaeus* agglutinin-1.) More than 90% of cultured TECs in a colony expressed p63 (Supplementary Fig. 1a), a transcription factor critical for maintenance of the proliferative potential of TECs[17]. We investigated whether clonogenic TECs had a keratin pattern indicative of a cortical (K8/18) or a medullary (K5/14) identity. Immediately after sorting, 60% of the $EpCAM^+$ cells stained positively for K5/14, whereas 20% were K8/18 positive; the remainder were double positive (Fig. 1c). After a week of cultivation, 77% of the colonies only contained $K5/14^+$ cells, whereas others contained only $K8/18^+$ cells (7%) or double positive cells (16%). After serial passaging, less than 2% of the cells were positive for K8/18, while all were positive for K5/14, a situation maintained in serial cultivation (Supplementary Fig. 1a).

These data indicate that clonogenic TECs originate from both the cortex and the medulla but that serial cultivation predominantly selected for a medullary phenotype. Clonogenic TECs were then

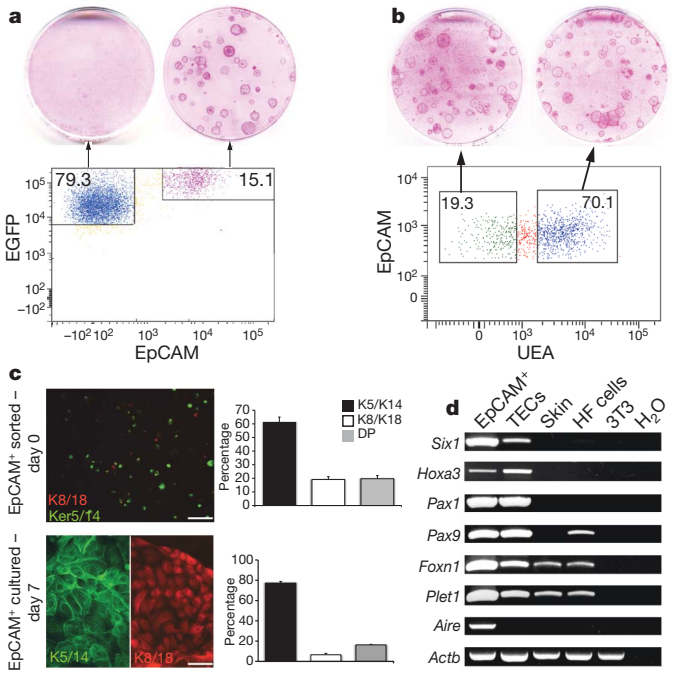

Figure 1 | Clonogenic TECs maintain thymic identity in culture. a, $EpCAM^+$ TECs can form colonies in culture. Fluorescence-activated cell sorting (FACS) analysis illustrates the distribution of $EpCAM^+$ and $EpCAM^-$ cells from E17 $EGFP^+$ thymi. Only a fraction of $EpCAM^+$ sorted cells (0.5%) formed colonies (50 cells out of 10^4 sorted cells). **b**, Clonogenic cells derive from both $EpCAM^+UEA^+$ and $EpCAM^+UEA^-$ populations. FACS plot shows the distribution of UEA^+ and UEA^- cells in the $EpCAM^+$ sorted cells. **c**, $EpCAM^+$ sorted cells were cytocentrifuged and immunostained for K5/14 and K8/18 as were 7-day-old colonies (scale bars, 50 μm). Percentage of TEC expressing K5/14, K8/18 or both (DP) was evaluated in three independent experiments. Data are mean ± s.d. **d**, Cultured $EGFP^+$ TECs maintain thymic identity. $EpCAM^+$ TECs were sorted and mRNAs extracted, as were cultured $EGFP^+$ TECs (embryonic day 16, E16, P1 and P7), skin (P0) and cultured hair follicle multipotent stem cells (P5). Transcripts were detected by RT–PCR (35 cycles) in EpCAM-sorted thymic cells and in subcultured TECs.

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passaged every six or seven days while maintaining 10–20% colony-forming efficiency over passages. We randomly cloned[20,21] 100–150 single TECs from first or second passage cultures. Cells cloned with an efficiency of 15–20%, and could be expanded for more than 50 doublings (Supplementary Fig. 1b) while maintaining a normal diploid karyotype ($2n = 42$). Most importantly, cultured TECs expressed several genes important for TEC identity including key transcriptions factors (Eya1, Six1, Pax1, Pax9, Foxn1 and Hoxa3) and *Plet-1* (placental-expressed transcript-1) encoding the cell surface antigen recognized by the monoclonal antibody MTS24 (ref. 22; Fig. 1d). MHC-class II and Aire expression were undetectable in cultured TECs (as in hair follicle stem cells) by immunochemistry. Colonies of TECs had a different phenotype from colonies of hair follicle stem cells (Supplementary Fig. 1c), even if they expressed markers of terminal epidermal differentiation—such as involucrin or LEKTI, a serine protease inhibitor encoded by *Spink5* (Supplementary Fig. 1d), and several other genes linked to epidermal, mucosal or hair follicle differentiation—as revealed by PCR with reverse transcription (RT–PCR) analysis (Supplementary Fig. 1e and 1f).

We investigated the ability of cultured TECs to contribute to thymic morphogenesis using a whole-organ re-aggregation assay[19,23–26] (Fig. 2a). Implanted aggregates consistently organized in structures that closely resembled a thymus (Fig. 2b), containing $CD4^+CD8^+$ positive lymphocytes of mouse origin within an epithelial network, even if the donor carrier cells were of rat origin (Fig. 2b), indicating that the implants had some thymic functionality as expected[23]. Clusters of $EGFP^+$ cells were identified in several areas and were predominantly localized in the medulla structure of the reconstituted thymi (Fig. 2b). A few $EGFP^+$ cells were also identified in other areas, in particular in the network lining the edge of a lobula (Fig. 2b); interestingly, the latter cells were $K5/14^-$ and $K8/18^-$. Clonal cultured TECs also consistently integrated in the medullary-like area (15/24 transplants) (Supplementary Table 1). MHC-class II expression was evident in the cultivated TECs engrafted in reconstituted thymi when revealed by an antibody specific to rat MHC-class II and confocal microscopy (Fig. 2c). Aire expression was also readily detected in some, but not all, GFP-labelled TECs (Fig. 2c). Most importantly, stem cells isolated from hair follicles, footpad, vagina and oesophagus only formed cyst-like structures containing limited number of $EGFP^+$ cells (Fig. 2d and Supplementary Table 1) and never expressed MHC-class II when incorporated in reconstituted thymi (not shown).

We then tested the capacity of clonogenic TECs to participate in the formation of hair follicles and epidermis in a short-term reconstitution assay[27] (Supplementary Fig. 2a). After 2–3 weeks, aggregates had generated $EGFP^+$ epidermis and hair follicles with a 10% frequency when cells were taken from thymus but with a 100% frequency when cells were from hair follicles (Supplementary Fig. 2b). Most importantly, $EGFP^+$ TECs were detected in all epidermal and hair follicle layers (Supplementary Fig. 2c), clearly indicating that clonogenic TECs were able to generate skin lineages in response to skin morphogenetic signals. We next investigated if TECs could participate in long-term hair follicle renewal using a functional assay developed for multipotent stem cells of the hair follicle[28] (Fig. 3a). Long-term participation of $EGFP^+$ TECs in epidermal renewal and hair follicle regeneration (hair cycle) was observed for several months in 18% of the grafts (10 of 56) in 10 independent transplants (Fig. 3b and Supplementary Table 2); cultured multipotent hair follicle stem cells (HF) and tracheal epithelial cells (pseudo-stratified epithelium) were used as positive and negative controls, respectively. Next, we recovered $EGFP^+$ colony-forming cells from four of six independent long-term skin grafts (Supplementary Fig. 3), indicating that thymus-derived epithelial cells could retain growth capability for a long time in a skin microenvironment. These skin-recovered cells (subsequently referred as sr1-TECs) were cultured for three to four passages before being transplanted into developing mouse skin. They were involved in epidermal renewal and were incorporated at a high frequency (23

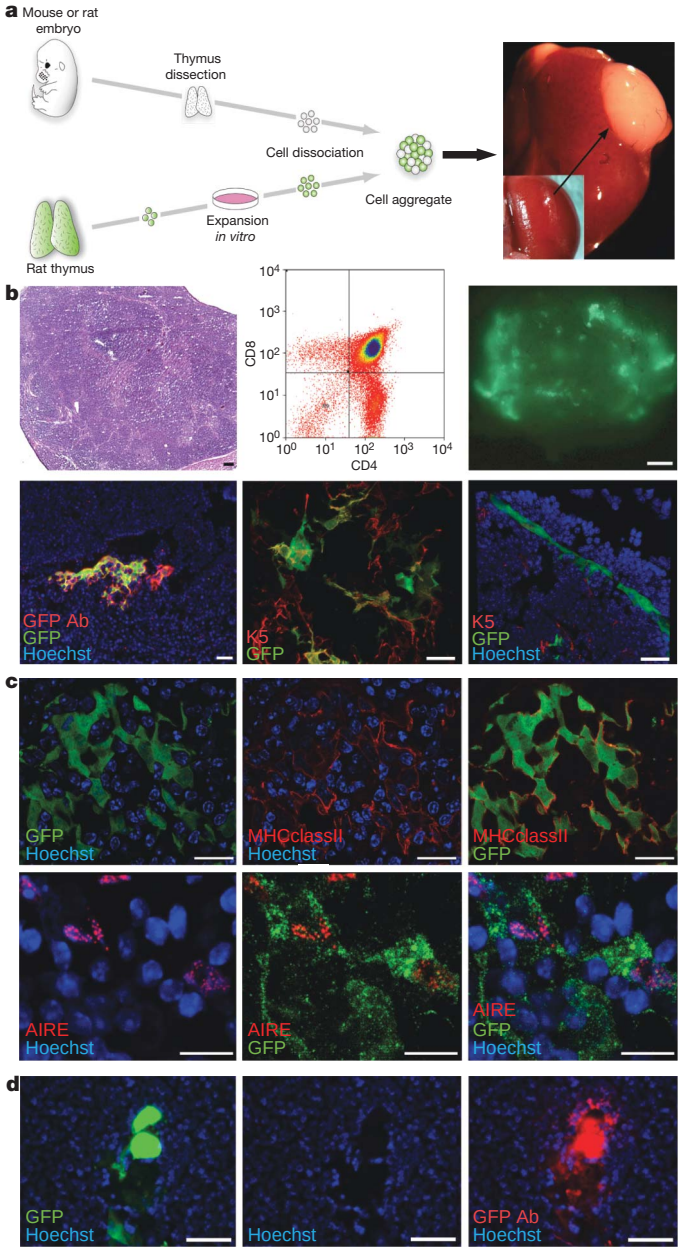

Figure 2 | Cultured thymic epithelial cells can incorporate into a thymic network and express MHC class II and Aire. a, Schematic representation of the thymic aggregation assay. Right panel, morphology of a chimaeric rat/mouse aggregate at grafting (insert) and 5 weeks post-transplantation under the kidney capsule of an athymic mouse. **b**, Incorporation of cultured $EGFP^+$ TEC cells into thymic aggregates. Upper-left panel, histology showing medulla and cortical areas (haematoxylin/eosin staining); scale bar, 100 μm. Upper-middle panel, flow cytometric analysis of T cells recovered from a rat/rat thymus with the presence of mouse CD4 and CD8 single- and double-positive T cells. Upper-right panel, gross appearance of a chimaeric rat/rat thymic aggregate, showing several areas containing $EGFP^+$ TECs from clone PRE16Cl11; scale bar, 500 μm. Lower-left panel, $EGFP^+$ cells immunostained with anti-GFP antibody. Lower-middle panel, confocal microscopy demonstrating that most integrated $EGFP^+$ cells adopted a medulla phenotype ($K5^+$). Lower right panel, integrated $EGFP^+$ TECs are lining a thymic lobule and do not express K5. Scale bars, 20 μm. **c**, High-resolution confocal microscopy demonstrating that $EGFP^+$ cells integrated into a 3D thymic network express MHC-class II and Aire. Upper panels, co-localization of rat MHC-class II (red) and $EGFP^+$ (green); scale bar, 20 μm. Lower panels, co-localization of the nuclear protein Aire (red) and EGFP (green); scale bar, 10 μm. **d**, Cultured HF multipotent stem cells do not integrate into a developing thymic microenvironment. Immunostaining against GFP protein (red) evidences $EGFP^+$ (green) area composed mostly of enucleated cells; scale bar, 25 μm. Nuclei are counterstained with Hoechst 33342 (blue).

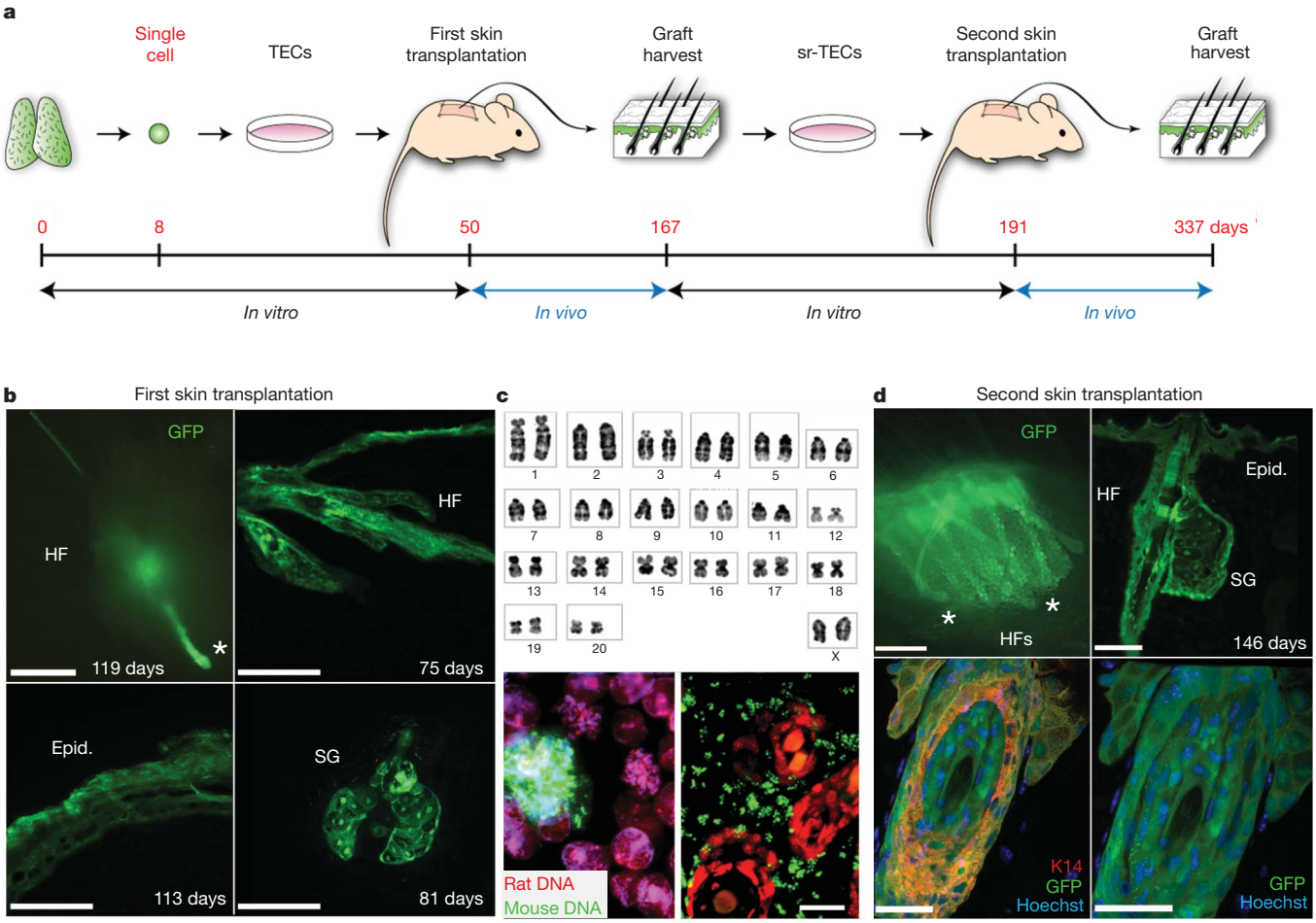

Figure 3 | Thymic epithelial cells have skin potency. **a**, Schematic representation of a serial-transplantation strategy. **b**, Cultured EGFP$^+$ TECs contribute to sebaceous glands (SG), epidermis and cycling HFs for several months. EGFP$^+$ TECs contribute to HF (clone PRP7Cl10 119 days post-transplantation (PT), scale bar, 500 μm), to HF and epidermis (clone PRE16Cl11, 75 days PT), to epidermis (clone PRE16Cl11, 113 days PT) and to sebaceous glands (clone PRP1Cl11, 81 days PT). Scale bars, 100 μm. Asterisk indicates location of hair bulb. **c**, EGFP$^+$ cells were isolated from the grafts and cultured again; sr1-TECs have a diploid karyotype ($n = 42$, XX). Lower-left panel, FISH of sr1-TECs labelled with a rat probe (orange) and a mouse probe (green), excluding fusion between mouse and rat cells. Nuclei are counterstained with Hoechst 33342 (blue). Lower-right panel, FISH on a cryosection (8 μm) of a skin serial transplant with a sr1-TEC clone: rat probe (orange) and mouse probe (green) show rat nuclei in HF structures and excluded *in vivo* fusion between donor rat and recipient mouse cells. FISH probes are detailed in Supplementary Information. Scale bar, 25 μm. **d**, Cultured sr1-TECs contributed to several EGFP$^+$ HF, SG and epidermis (146 days PT); asterisk locates hair bulbs; scale bars, 500 and 50 μm. At higher magnification (lower panels) a transversal section of a HF shows contribution of cultured TECs to all layers of the HF structure. Scale bar, 20 μm.

of 24) into hair follicles that cycled for more than 100 days (Fig. 3d and Supplementary Table 2).

Unexpectedly, the sr1-TECs also renewed the epidermis in sharp contrast to bona fide HF multipotent stem cells that can only generate epidermis for 3 weeks or after injury in this assay[28]. To eliminate the possibility that transplanted rat TECs had fused with cells of the recipient mouse, we performed a two-colour fluorescent *in situ* hybridization (FISH) using mouse (green) and rat (orange) genomic DNA as probes (Fig. 3c); no double-labelled cells were detected, thus eliminating fusion. Furthermore, a karyotype analysis revealed that 93% of the EGFP$^+$ TECs cultured after the first round of skin transplantation had a normal rat chromosomal count (Fig. 3c). We next examined the ability of sr1-TECs to integrate into a thymic epithelial network in our reconstitution assay (18 of 28) (Supplementary Table 1). sr1-TECs did not perform homogeneously; one clone (no. 57) behaved similarly to HF cells and it did not integrate into a thymic microenvironment, whereas two other clones (nos 217 and 218) integrated into the thymic epithelial network and expressed MHC class II antigens and Aire (Fig. 4a and b). In addition, one clone (no. 217) gave also rise to stratified epithelium and sebaceous glands (Fig. 4b). Together, these data suggest that the recovered clones were primed towards the HF fate to different extents.

We next performed a genome-wide expression screen on clonogenic TECs, sr1-TECs and clones recovered after a second skin transplantation (subsequently referred as sr2-TECs, Supplementary Fig. 3). Individual clonal populations behaved differently; after the first round of transplantation, some sr1-TECs clustered closely with TECs while others clustered close to hair follicles; this pattern was maintained after the second round of skin transplantation, indicating that additional exposure to skin microenvironmental cues had little impact on reprogramming (Fig. 4c). This pattern correlated with functional capacity: sr1-TEC no. 218 efficiently integrated into the thymic network in the reconstitution assay and clustered close to thymic epithelial cells; in contrast, sr1-TEC no. 217, which both incorporated into the thymic network and generated epidermis and sebaceous glands in the thymic assay, clustered closer to hair follicle; and sr1-TEC no. 57, which did not integrate into thymus clustered very close to hair follicle (Fig. 4c). Accordingly, robust change in expression of genes was observed, including downregulation of transcription factors important to TECs (for example, *Pax1*, *Pax9* and *Eya1*), and upregulation of epidermal markers (for example, Lhx2, galectin 7 and claudins; Supplementary Tables 3 and 4). RT–PCR demonstrated that thymus-specific genes, like *Six1*, *Pax9*, *Hoxa3*, *Foxn1* and *Plet-1*, were consistently expressed in cultured sr1-TECs (not shown), whereas

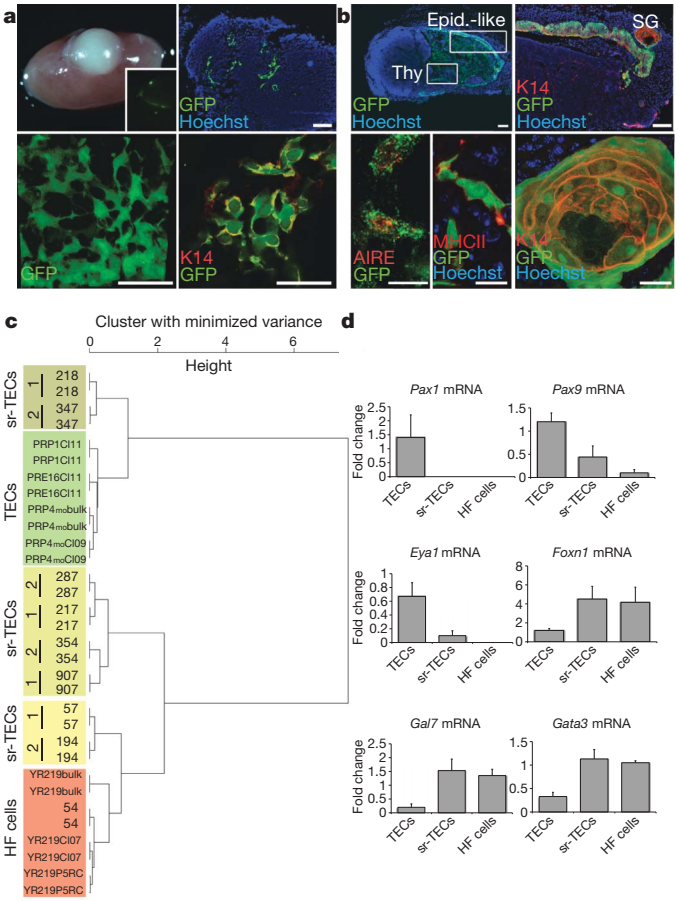

Figure 4 | Fate of skin-recovered thymic epithelial cells. a, **b**, sr-TEC clones display a non-homogeneous behaviour when incorporated into thymic aggregates. **a**, sr1-TECs no. 218 integrated into the epithelial thymic network and contributed to the medullary compartment; scale bar, 100 μm. Lower panels, several EGFP$^+$ cells (green) expressed K14; scale bars, 25 μm. **b**, Upper panels, sr1-TECs no. 217 integrated into a thymic network, but also generated an epidermis-like structure (Epid-like or ORS-like structure) and sebaceous glands (SG); K14 immunostaining (red); scale bars, 100 μm. Lower right panel, K14 staining of SG; scale bar, 20 μm. A third clone (clone 57) did not integrate at all (not shown). Lower-left and middle panels, expression of Aire and rat MHC-class II antigens; scale bars, 10 μm. **c**, Microarray analysis of cultured TECs and HF cells before and after skin transplantation; cluster with minimized variance based on 119 transcripts differentially expressed between HF cells and TECs ($P<0.001$ and a fold change higher than 4). sr-TECs clustered closer to TECs, to HF or very close to hair follicle after the first transplantation (1), and maintained the same pattern after the second skin transplantation (2). Cluster correlates with the capacity of the sr-TECs to incorporate into a thymic network. **d**, Relative fold changes for *Pax1*, *Pax9*, *Eya1*, *Foxn1*, *Gal7* and *Gata3* transcripts (Q-PCR). Data show mean ± s.e.m. (TECs: $n = 3$; sr-TECs: $n = 4$ and HF cells: $n = 2$). sr-TECs represent the mean of sr1- and sr2-TECs.

Pax1 expression was undetectable, a finding confirmed by quantitative PCR (Fig. 4d).

Our results demonstrate that embryonic and postnatal thymi contain a subpopulation of clonogenic TECs that can function as epidermal stem cells and as multipotent hair follicle stem cells when exposed to an inductive skin microenvironment, unambiguously demonstrating an increase in potency[13]. Recent reports indicate that cortical and medullary TECs derive from a common bipotent precursor[18,29] or that the thymus may contain a small population of epithelial stem cells[17,30]; our results favour the latter, as suggested by the extensive self-renewal of cultured TECs and their capacity to incorporate into a three-dimensional epithelial network while expressing MHC-class II and Aire. Several mechanisms could explain why TECs can acquire skin potency. First, TECs may have an inherent capability to form skin, as revealed by our assay, as they naturally express markers of skin differentiation *in vivo*. This would suggest the existence of a generic program of stratification and that skin determination is independent of primary germ line origin, as TECs are of endodermal and not of ectodermal origin. The question then arises as to why TECs need a stratification program while forming a complex three-dimensional network, the organization of which is strikingly different from stratified epithelia. Second, TECs may improve their ability to become multipotent keratinocyte stem cells following microenvironmental reprogramming. The robust changes in gene expression in response to exposure to skin morphogenetic signals favour this hypothesis, as does the fact that the TECs, in which the transcriptome clusters closest to hair follicle stem cells, have lost their thymic epithelial competence. Third, TECs may express a unique epithelial program, spanning from simple to stratification, which primes them for subsequent reprogramming by morphogenetic signals. Whether TECs can respond to a microenvironment other than skin will be of interest.

METHODS SUMMARY

EGFP Sprague–Dawley rats (green rat CZ-004'' SDTgN (act-EGFP)obsCZ-004) were from Japan SLC[14]. Hair follicle and thymic epithelial cells were cultured onto a feeder layer of lethally irradiated 3T3-J2 cells as described[21,28]. Single cells were isolated as described[20] and clones were expanded once a week. Thymic aggregates were performed as described[26]. Briefly, thymi were dissected from either E13.5/E14.5 wild-type mouse embryos or E15/E16 rat embryos and dissociated into a single-cell suspension and used to aggregate cultured TECs obtained from EGFP rats, in approximately 1:4 ratio (cultured TECs/donor carrier cells),and then implanted under the kidney capsule of athymic mice. Skin aggregates were performed as described[27]. Briefly, mass or clonal cultures of TECs were obtained from embryos (PRE16) or from newborn EGFP rats (PRP1) and were aggregated *ex vivo* with skin cells obtained from E14.5 wild-type mouse embryos and implanted under the kidney capsule of athymic mice. Cultured hair follicle multipotent stem cells were used as a control. For long-term skin reconstitution assay, cultured TECs or hair follicle multipotent stem cells were grafted as described[28]. Gene profiling were performed at the Lausanne DNA Array Facility (DAFL) using rat Affymetrix chips (Rat Gene ST 1.0 Array). Chrombios (Raubling, Germany) performed karyotyping and FISH analyses. Detailed methods and procedures including primers for PCR analysis, antibodies for flow cytometry and immunostaining are provided in Supplementary Information.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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1. Manley, N. R. & Blackburn, C. C. A developmental look at thymus organogenesis: where do the non-hematopoietic cells in the thymus come from? *Curr. Opin. Immunol.* **15**, 225–232 (2003).
2. Boehm, T. Thymus development and function. *Curr. Opin. Immunol.* **20**, 178–184 (2008).
3. Anderson, G., Lane, P. J. & Jenkinson, E. J. Generating intrathymic microenvironments to establish T-cell tolerance. *Nature Rev. Immunol.* **7**, 954–963 (2007).
4. van Ewijk, W. *et al.* Thymic microenvironments, 3-D versus 2-D? *Semin. Immunol.* **11**, 57–64 (1999).
5. Kyewski, B. & Klein, L. A central role for central tolerance. *Annu. Rev. Immunol.* **24**, 571–606 (2006).
6. Abramson, J., Giraud, M., Benoist, C. & Mathis, D. Aire's partners in the molecular control of immunological tolerance. *Cell* **140**, 123–135 (2010).
7. Blackburn, C. C. *et al.* One for all and all for one: thymic epithelial stem cells and regeneration. *Trends Immunol.* **23**, 391–395 (2002).
8. Holländer, G. *et al.* Cellular and molecular events during early thymus development. *Immunol. Rev.* **209**, 28–46 (2006).
9. Mathis, D. & Benoist, C. Aire. *Annu. Rev. Immunol.* **27**, 287–312 (2009).
10. Laster, A. J., Itoh, T., Palker, T. J. & Haynes, B. F. The human thymic microenvironment: thymic epithelium contains specific keratins associated with early and late stages of epidermal keratinocyte maturation. *Differentiation* **31**, 67–77 (1986).
11. Heid, H. W., Moll, I. & Franke, W. W. Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. I. Human and bovine hair follicles. *Differentiation* **37**, 137–157 (1988).
12. Hale, L. P. & Markert, M. L. Corticosteroids regulate epithelial cell differentiation and Hassall body formation in the human thymus. *J. Immunol.* **172**, 617–624 (2004).
13. Smith, A. A Glossary of stem-cell biology. *Nature* **441**, 1060 (2006).

14. Ito, T., Suzuki, A., Imai, E., Okabe, M. & Hori, M. Bone marrow is a reservoir of repopulating mesangial cells during glomerular remodeling. *J. Am. Soc. Nephrol.* **12,** 2625–2635 (2001).
15. Ronfard, V., Rives, J. M., Neveux, Y., Carsin, H. & Barrandon, Y. Long-term regeneration of human epidermis on third degree burns transplanted with autologous cultured epithelium grown on a fibrin matrix. *Transplantation* **70,** 1588–1598 (2000).
16. Sun, T. T., Bonitz, P. & Burns, W. H. Cell culture of mammalian thymic epithelial cells: growth, structural, and antigenic properties. *Cell. Immunol.* **83,** 1–13 (1984).
17. Senoo, M., Pinto, F., Crum, C. P. & McKeon, F. p63 Is essential for the proliferative potential of stem cells in stratified epithelia. *Cell* **129,** 523–536 (2007).
18. Bleul, C. C. *et al.* Formation of a functional thymus initiated by a postnatal epithelial progenitor cell. *Nature* **441,** 992–996 (2006).
19. Hamazaki, Y. *et al.* Medullary thymic epithelial cells expressing Aire represent a unique lineage derived from cells expressing claudin. *Nature Immunol.* **8,** 304–311 (2007).
20. Barrandon, Y. & Green, H. Three clonal types of keratinocyte with different capacities for multiplication. *Proc. Natl Acad. Sci. USA* **84,** 2302–2306 (1987).
21. Rochat, A., Kobayashi, K. & Barrandon, Y. Location of stem cells of human hair follicles by clonal analysis. *Cell* **76,** 1063–1073 (1994).
22. Depreter, M. G. *et al.* Identification of Plet-1 as a specific marker of early thymic epithelial progenitor cells. *Proc. Natl Acad. Sci. USA* **105,** 961–966 (2008).
23. Anderson, G., Jenkinson, E. J., Moore, N. C. & Owen, J. J. MHC class II-positive epithelium and mesenchyme cells are both required for T-cell development in the thymus. *Nature* **362,** 70–73 (1993).
24. Gill, J., Malin, M., Hollander, G. A. & Boyd, R. Generation of a complete thymic microenvironment by MTS24(+) thymic epithelial cells. *Nature Immunol.* **3,** 635–642 (2002).
25. Bennett, A. R. *et al.* Identification and characterization of thymic epithelial progenitor cells. *Immunity* **16,** 803–814 (2002).
26. Sheridan, J. M., Taoudi, S., Medvinsky, A. & Blackburn, C. C. A novel method for the generation of reaggregated organotypic cultures that permits juxtaposition of defined cell populations. *Genesis* **47,** 346–351 (2009).
27. Moscona, A. Rotation-mediated histogenetic aggregation of dissociated cells. A quantifiable approach to cell interactions *in vitro*. *Exp. Cell Res.* **22,** 455–475 (1961).
28. Claudinot, S., Nicolas, M., Oshima, H., Rochat, A. & Barrandon, Y. Long-term renewal of hair follicles from clonogenic multipotent stem cells. *Proc. Natl Acad. Sci. USA* **102,** 14677–14682 (2005).
29. Rossi, S. W., Jenkinson, W. E., Anderson, G. & Jenkinson, E. J. Clonal analysis reveals a common progenitor for thymic cortical and medullary epithelium. *Nature* **441,** 988–991 (2006).
30. Rodewald, H. R. Thymus organogenesis. *Annu. Rev. Immunol.* **26,** 355–388 (2008).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions P.B., C.C.B. and Y.B. contributed to the design of the experiments and the interpretation of the results, S.C., A.W.A., A.F. and P.B. performed experiments, and Y.B. and P.B wrote the paper.

Author Information Raw and normalized microarray data are accessible through the NCBI Gene Expression Omnibus public (http://www.ncbi.nlm.nih.gov/geo) data base (series record GSE21686). Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to Y.B. (Yann.Barrandon@epfl.ch).

METHODS

Animals. Sprague–Dawley rats, which constitutively expressed EGFP (green rat CZ-004'' SDTgN (act-EGFP)obsCZ-004), were supplied by Japan SLC[14]. Wild-type Sprague–Dawley (OFA) rats were obtained from Iffa Credo. Athymic (Swiss $Nu^{-/-}$) and OF1 mice were supplied by Charles River Breeding Laboratories. All animals were maintained in a 12 h light cycle providing food and water *ad libitum*. Rats were killed by CO_2 inhalation, whereas mice were killed by intra-peritoneal (i.p.) injection of pentobarbital. Experiments were conducted in accordance with the EU Directive (86/609/EEC) for the care and use of laboratory animals and that of the Swiss Confederation. Mating of adult female and male rats (age >11 weeks) was carried out overnight. Time-pregnant rats were killed by CO_2 inhalation and uteri with embryos were removed by dissection.

Cell dissociation. Thymic lobes were removed from embryos at E16 and E17 and from postnatal rats at P0, P1, P7, P12, P32 and P137. Dissected thymic lobes were harvested and kept in cold Hank's Balanced Salt Solution (HBSS, Gibco–Invitrogen) complemented with 10% Fetal Bovine Serum (FCS, Gibco–Invitrogen) during dissection. Single lobes were placed in individual 35 mm Petri dishes and washed in HBSS without Ca^{2+} and Mg^{2+} twice before being dissociated in 0.05% trypsin solution containing 0.1% EDTA at 37 °C. Skin grafts containing GFP-positive hair follicles were carefully dissected from the back of recipient nude mice and dissociated in 30 ml of 0.05% trypsin containing 0.1% EDTA at 37 °C with magnetic stirring for 60 to 120 min.

Cell culture. Hair follicle and TECs were cultured onto a feeder layer of lethally irradiated 3T3-J2 cells as described[21,28]. 3T3 cells were cultured in Dulbecco-Vogt modification of Eagle's Medium (DMEM, Gibco–Invitrogen) supplemented with 10% bovine serum (BS) (Hyclone), fed every 2–3 days and serially passed once a week for a maximum of 12 weeks. 3T3 cells used as feeder layer were irradiated (60 Gy) and plated at a density of 2.5×10^4 cells cm^{-2}. Epithelial cells were cultured in cFAD medium consisting of a 3:1 mixture of DMEM and Ham's F-12 medium (Gibco–Invitrogen). Supplements were as follows: 10% FCS (Gibco), hydrocortisone 0.4 μg ml^{-1} (Calbiochem, VWR Int.), 10^{-6} M cholera toxin (Sigma,), 5 μg ml^{-1} insulin (Sigma), 2×10^{-9} M 3,3′,5-triiodo-L-thyronin (T3) (Sigma). Recombinant human epidermal growth factor rhEGF (QED) was added at the first feeding (10 ng ml^{-1}). All cultures were incubated at 37 °C in a 10% CO_2 atmosphere, and the medium was changed twice a week. Cultures were passaged once a week.

Single cell isolation. Single cells were isolated as described[31]. Briefly, individual cells were aspirated into a Pasteur pipette under a Zeiss Axiovert inverted microscope using a 10× objective and immediately inoculated into a 35 mm Petri dish already containing lethally irradiated 3T3 cells. Cultures were fed every 3–4 days with cFAD medium. Clones were passaged and expanded once a week.

Colony-forming efficiency. Hundred cells were plated onto a 60 mm Petri dish containing lethally irradiated 3T3 cells and cultured for 12 days as described before they were fixed in formalin and stained in 1% rhodamine-B. Colonies were scored under a dissecting microscope.

Thymic aggregates. Thymic aggregates were obtained following a compaction protocol[26], modified as follows. Briefly, E13.5/E14.5 mouse embryos (OF1) or E16/E17 rat embryos (SD) were harvested from the uteri under sterile conditions, transferred in 100 mm Petri dishes containing 10% BS-DMEM medium and stored on ice. Thymic lobes were removed from the embryonic chests and immerged into cold HBSS supplemented with 10% FCS. Subsequently, the lobes were washed in HBSS without Ca^{2+} and Mg^{2+} and transferred into a solution containing collagenase/dispase (1 mg ml^{-1}) and 0.05% DNase for 30 min. The lobes were then dissociated in a trypsin solution (0.05%) containing 0.1% EDTA until a single cell suspension was obtained. Embryonic thymic cells were mixed with cultured thymic cells at different ratios: 6–8 dissociated embryonic lobes with 10^5 to 2×10^5 cultured TECs or HF cells expressing GFP; 10^5 mouse fibroblasts were added as carrier cells as described[23]. To obtain aggregates, cells were aspirated into a 200 μl pipette and centrifuged at 1,300 r.p.m. for 3 min (ref. 26). Aggregates were then transferred on a 0.8 μm Isopore membrane filter (Millipore) and incubated at 37 °C for 24 h before being grafted under the kidney capsule of athymic mice.

Skin aggregates. Aggregates were obtained as described[27] with modifications. Briefly, the skin was dissected under sterile conditions from the back of E13.5 and E14.5 mouse embryos and dissociated in 0.05% trypsin containing 0.1% EDTA at 37 °C until a single cell suspension was obtained. Dissociated cells were filtered through a 70 μm cell-strainer and counted. 4×10^5 skin cells were mixed with $(1–2) \times 10^5$ cultured TECs or HF cells and plated in individual 96-well V-bottom plate. Cells were aggregated in the plate by centrifugation (1,300 r.p.m., 10 min). Aggregates were kept at 37 °C in a 10% CO_2 atmosphere for 24 h before being grafted under the kidney capsule of athymic mice.

Skin morphogenetic assay. Cultured TECs or hair follicle multipotent stem cells were grafted as described[28]. Briefly, a piece of skin was obtained from a newborn mouse (OF1) and incubated in 2% EDTA at 37 °C for 120 min. After rinsing in medium supplemented with 10% BS, the epidermis was gently separated from the dermis to form a small pocket with the help of a 30-gauge needle. Donor cells (5×10^5) were then carefully injected into the pocket and allowed to attach at room temperature for 1 h. Grafts were kept overnight at 4 °C before being transplanted onto the back of athymic mice with their dermal side facing the mouse fascia, stitched in place and covered with a skin flap to prevent drying. Flaps underwent necrosis or were manually opened a few days later, so the graft became air-exposed. In rare cases, flaps were removed after 12–15 days. Transplanted cells remained on athymic mice for the duration of the experiment, usually several months.

RT–PCR. Total RNA was isolated from rat TE cultured cells or from dissected thymic lobes using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Total RNA was extracted using TRIzol reagent (Invitrogen) and amplified using the OneStep RT–PCR kit (Qiagen) or reverse transcribed using the Superscript III enzyme with random primers (Invitrogen) and amplified with the GoTaq PCR reagent kit (Promega) for 35 or 39 cycles. 1 μg of total RNA was reverse-transcribed as detailed in the SuperscriptTM III reverse transcriptase kit (Invitrogen). Random primers were used at 100 ng ml^{-1} (Invitrogen), the dNTPs mix at 0.5 mM (Qiagen) and the RNasin RNase inhibitor at 2 U ml^{-1} (Promega). PCR products were resolved by agarose gel electrophoresis, stained with ethidium bromide, and visualized under ultraviolet light. β-actin control for equal loading was used throughout the experiments. Primers are listed in Supplementary Table 3 and were designed to span at least one intron using Primer3 software.

Quantitative PCR. For Q-PCR, cDNAs were diluted 10 times and 1 μl was ampli?ed with the Light-Cycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics), in a 12.5 μl total reaction volume; 1.15 μl of the FastStart Reaction mix SYBR Green I, 4 mM $MgCl_2$ and 0.5 μM of each primer were included in the reaction. Rat primers were designed using the Light-Cycler Probe Design program from Roche (temperature of annealing set at 60 °C) and Microsynth. The Q-PCR program consisted of hot start enzyme activation at 95 °C for 15 min, 40 cycles of amplification at 95 °C for 10 s, 55 °C or 60 °C for 5 s and 72 °C for 6 s. Finally, to obtain the melting curve, the analysis at 65 °C for 15 min was followed with a cooling cycle to reach 40 °C for 30 s. For the data analysis, the rat hypoxanthine-guanine phosphoribosyltransferase (HPRT) housekeeping gene was used as internal control. Induction values were calculated using the Roche RelQuant analysis software. List of primers is given in Supplementary Table 4.

Microarrays. Quantitative analysis of RNA expression was performed using Affymetrix gene chip cDNA microarrays (Affymetrix). Cultivated cells sorted on the basis of GFP expression were used to prepare total RNA using the RNeasy Mini Kit (Qiagen). RNA quality was measured using the Agilent Bioanalyzation system (Agilent Technologies), to ensure the integrity of the RNA. cDNA synthesis, hybridization to the Affymetrix GeneChip Rat Expression Array 230 2.0 (31,042 gene-level probe sets) or Rat Gene ST 1.0 Array (27,342 gene-level probe sets) and analysis was performed by the Lausanne DNA Array Facility (DAFL) using standard protocols. To identify differentially expressed genes from each group, *P* values were calculated using Bioconductor limma package[32] and probe sets with a false discovery rate[33] <0.05 were considered significant.

Immunostaining. Immunohistochemistry (IHC) and immunocytochemistry (ICC) were performed on tissue sections or cultured cells. For tissue sections, entire tissue was embedded in OCT compound (Tissue-Tek), frozen in −40 °C methyl-butane. Sections (8-μm) were cut on a Leica CM3000 cryostat. Cells were cultured onto glass coverslips. Tissues or cells were fixed in 4% paraformaldehyde. Aspeci?c sites were blocked with 5% goat serum/PBS (for extracellular epitopes) or 5% goat serum/PBS/0.1% triton (for intracellular epitopes). Tissues or slides were incubated with primary antibodies diluted in blocking buffer overnight at 4 °C. Secondary antibodies were Alexa-Fluor568 or Alexa-Fluor488 conjugated (Molecular Probes, Invitrogen) and used at 1:400 dilution. Nuclei were stained with Hoechst 33432 (10^{-6} M). The DABCO solution (Sigma) with glycerol (Merck) in PBS was used for mounting. The following primary antibodies were used: rabbit anti-keratin 5 (1:500; Abcam), rabbit anti-keratin 14 (1:500; Covance), mouse anti-LEKTI (1:100; Zymed), mouse anti-keratin 18 (1:100; Progen), mouse anti-p63 (1:50; Dako), mouse anti-involucrin (1:100; Lab Vision), guinea pig anti-keratin 8 (1:100; Neomarkers); mouse anti-MHCclassII (1:400; Abcam); rabbit anti-GFP (1:400; Abcam); rabbit anti-Ki67 (1:400; Abcam); rabbit anti-pankeratin (1:400; Dako); goat anti-Aire D-17 (1:100 Santa Cruz); rabbit anti-Aire M300 (1:100; Santa Cruz); chicken anti-GFP (1:500; Abcam); mouse anti-rat MHC Class II (1:200; eBiosciences).

Histology. Cryosections of thymic aggregates (8 μm) were stained with haematoxylin-eosin.

Karyotyping and FISH analyses. Chrombios (Raubling) performed karyotype and FISH analyses. Rat genomic DNA was labelled with carboxytetramethyl-rhodamine-dUTP (red fluorescence) by degenerate oligonucleotide primer

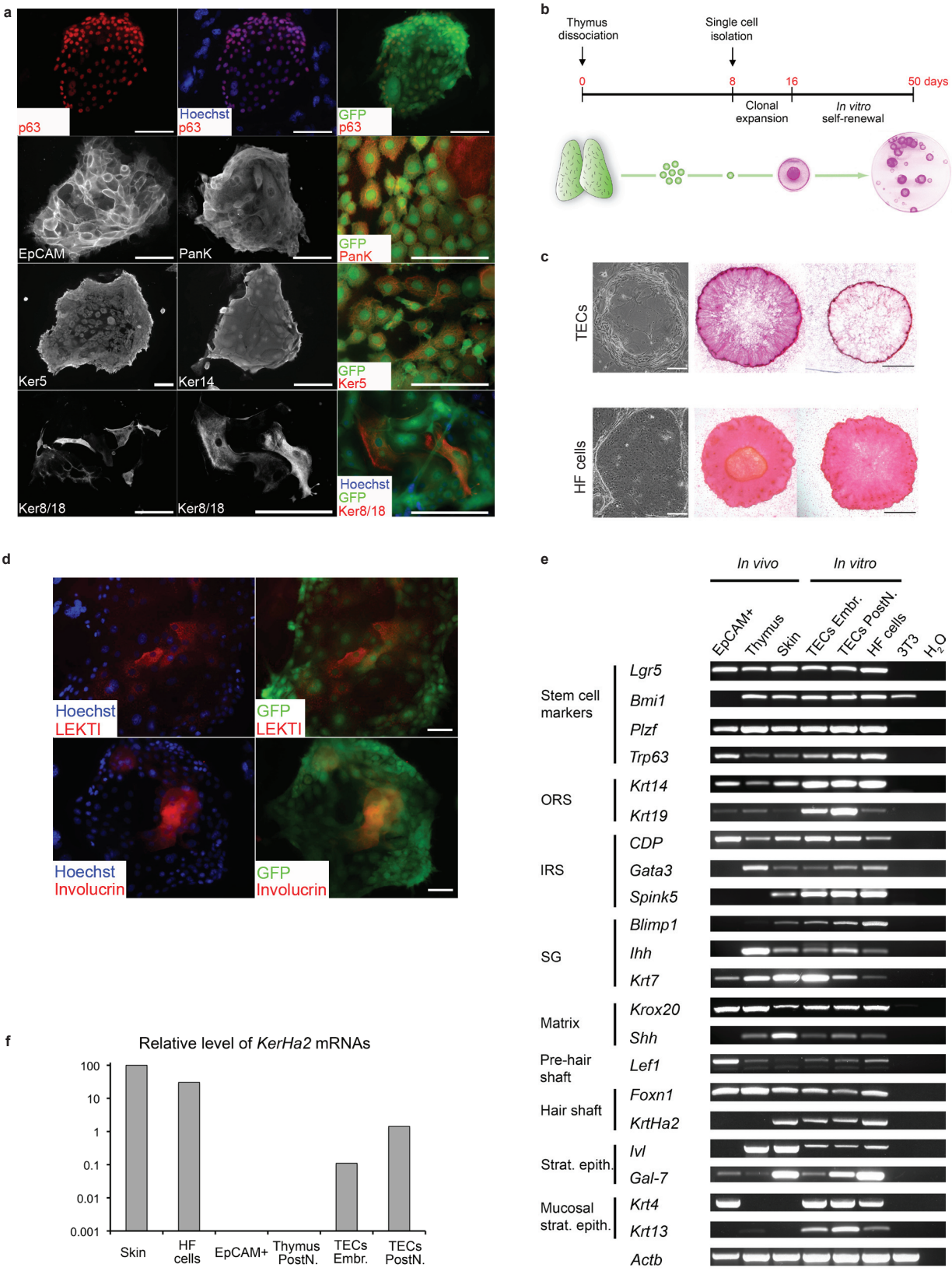
PCR, and mouse satellite DNA was labelled with FITC-dUTP (green fluorescence). 8 μm tissue sections were incubated at 50 °C for 1 h and then pepsin treated (200 μl 0.01 N HCl/2 μl pepsin (7.5 u μl^{-1})) at 37 °C for 12 min. Sections were washed in 2× SSC for 20 min, then dehydrated in graded ethanol and dried at 50 °C. For FISH, 3 μg of each mouse and rat genomic DNA were used for labelling by a standard nick translation procedure (90 min, 15 °C). Rat DNA was labelled with TAMRA-dUTP, and mouse DNA with FITC-dUTP at the concentration of 20 μM for each dUTP. 300 ng of each probe was combined, precipitated and resolved in 15 μl hybridization buffer (50% formamide, 2× SSC, 10% dextran sulphate). Probes and sections were denatured together on a hot plate (72 °C) for 8 min. Hybridization was performed at 37 °C in a humid chamber overnight. Sections were then washed in 2× SSC at room temperature for 10 min followed 70 °C for 1 min in 0.4× SSC/Tween, before they were stained with DAPI. Sections were examined with a Axioplan II fluorescence microscope (Zeiss) equipped with a black-and-white CCD camera. Image capture was performed with SmartCapture software (Digital Scientific) using appropriate fluorescence filters (ChromaTechnology).

Flow cytometry. Thymi were obtained from rats and dissociated into single cells as described above. Cells were resuspended in cold HBSS supplemented with 10% FCS and were kept on ice. Cells were centrifuged at 4 °C for 10 min at 1,500 r.p.m., resuspended in HBSS containing 10% FCS and blocked at 4 °C for 15 min. Cells were then incubated with a mouse anti-EpCAM antibody (1:100, Biovendor) for 30 min on ice, centrifuged at 1,100 r.p.m. for 10 min, washed in HBSS and resuspended in HBSS containing the AlexaFluor 647-conjugated goat anti-mouse IgG1 antibody (Molecular Probes, Invitrogen). Cells were finally incubated at 4 °C for 15 min before they were washed with ice-cold HBSS. Proper isotype control (mouse IgG1) was used as a negative control. Cells were resuspended in HBSS with 10% FCS, filtered using a 70-μm cell strainer (Millipore), and sorted by flow cytometry using a fluorescence-activated cell sorter (FACS) DIVA (BD Biosciences). Clean separation between $EpCAM^+$ and $EpCAM^-$ cell populations were confirmed by second FACS analysis. Dead cells were excluded by addition of propidium iodide (Sigma) and gating on the negative cells. Fluorescent staining for *Ulex europaeus* agglutinin I (UEA-1) was performed using biotinylated UEA-1 (1:800, Vector Laboratories) and secondary reagent was Streptavidin-PE/Cy7 (1:100; Biolegend).

Image acquisition and processing. Images were acquired under inverted fluorescence microscope Axiovert 200 or the Leica Sp2 confocal upright and inverted microscopes. Confocal images and three-dimensional reconstruction were processed using Imaris 6.2 software.

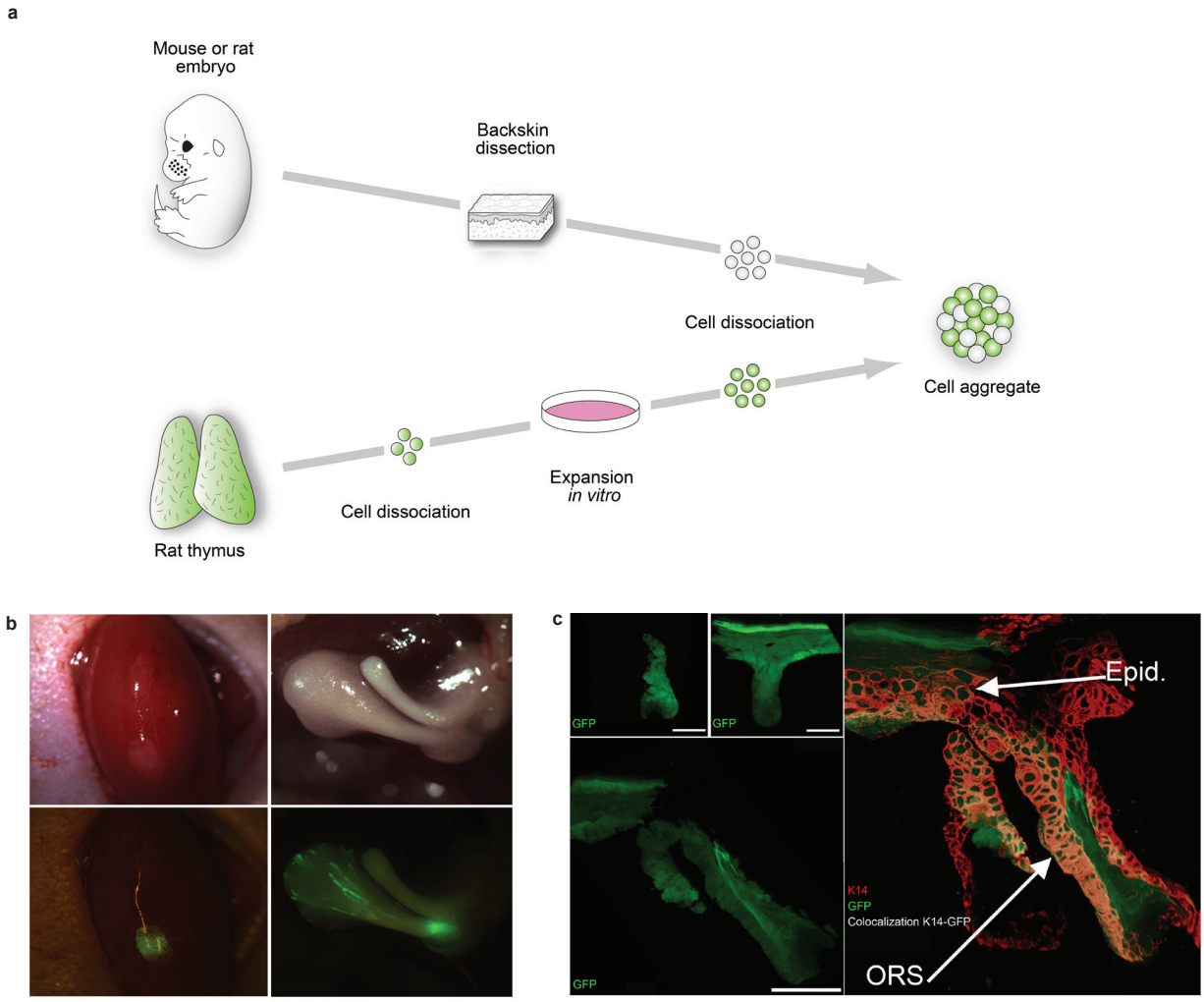
31. Barrandon, Y. & Green, H. Cell size as a determinant of the clone-forming ability of human keratinocytes. *Proc. Natl Acad. Sci. USA* **82**, 5390–5394 (1985).
32. Smyth, G. K. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* **3**, article 3 (2004).
33. Hochberg, Y. & Benjamini, Y. More powerful procedures for multiple significance testing. *Stat. Med.* **9**, 811–818 (1990).

SUPPLEMENTARY INFORMATION

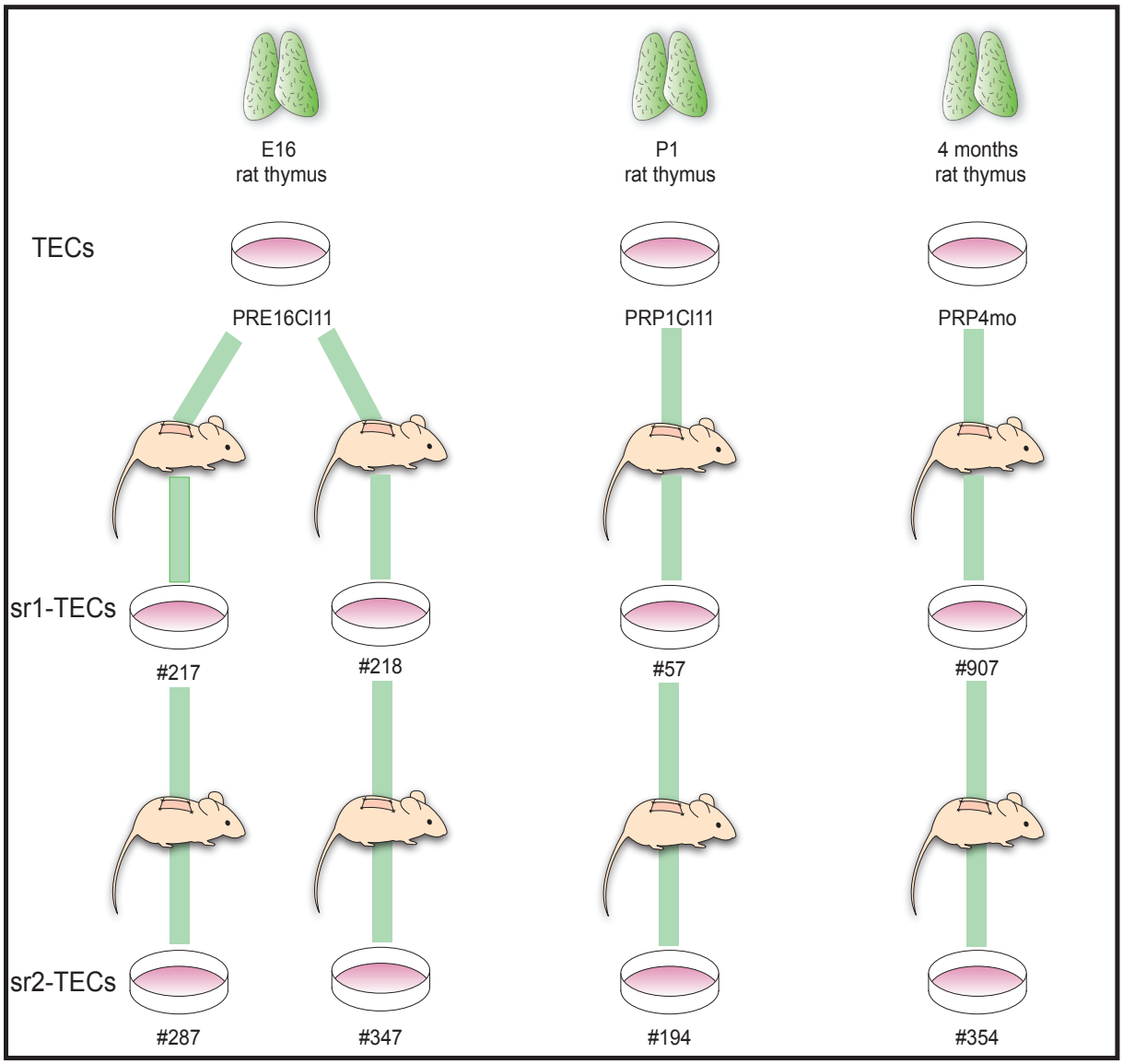


Supplementary Figure 1. Cultured thymic epithelial cells expressed markers of epidermal differentiation
a) Cultured TECs express epithelial markers. All TECs are positive for EpCAM and stained with a pan-keratin antibody; more than 90% of TECs express p63 after immunostaining, most express medulla markers K5/K14 and less than 2% express cortical markers K8/K18. Cells positive for K8/K18 also display a unique, elongated

morphology. Nuclei are counterstained with Hoechst 33342 (blue). Bars = 100μm. Cultured TECs express epithelial markers. All TECs are positive for EpCAM and stained with a pan-keratin antibody; more than 90% of TECs express p63 after immunostaining, most express medulla markers K5/K14 and less than 2% express cortical markers K8/K18. Cells positive for K8/K18 also display a unique, elongated morphology. Nuclei are counterstained with Hoechst 33342 (blue). Bars = 100μm. **b)** TECs can be cloned and serially passaged. **c**) Morphology of TECs colonies differs from that of hair follicles. Microscopic appearance of 6 day-old growing colonies under phase contrast and of 12 day-old colonies after Rhodamine B staining. The dark Rhodamine-stained colonies represent in average 70-90% of the colonies, while the light Rhodamine-stained colonies represent 10-30%. Bars = 200μm and 2.5mm. **d**) Cultured $EGFP^+$ TECs expressed involucrin and Lekti, markers of epidermal differentiation at the center of the colonies. Nuclei are counterstained with Hoechst 33342 (blue). Bars = 50μm. **e**) Uncultured TECs and cultured TECs expressed markers of hair differentiation. RNAs were extracted from rat skin (P0), rat $EPCAM^+$ sorted cells (E17) and rat postnatal thymus (4 weeks-old), TECs expanded in culture from both embryonic (E17) and postnatal thymi (P1), and HF multipotent stem cells (P5). RT-PCR (39 cycles) for *Bmi1*, *Plzf*, *p63* (stemness); *K5* and *K14*, (epidermal basal layer); *K14*, *K19* (HF outer root sheath - ORS); *K7*, *Indian HH*, *Blimp1* (sebaceous gland, SG); *Krox20* (matrix); *CDP*, *Gata3*, *Spink5* (HF inner root sheath - IRS); *Lef1* (pre-cortical); *Foxn1* and *KerHa2* (hair cortex); *involucrin* and *Gal-7* (epidermal terminal differentiation); *K4* and *K13* (mucosal differentiation). **f)** Q-PCR for RNA-expression level of *KerHa2* showing a 20-50 fold difference between HF and TECs (n=2). Cells were obtained from subcultures 3 to 5.



Supplementary Figure 2. Skin aggregate assays a) Schematic drawing of the skin aggregation assay. The progeny of a single clonogenic TEC obtained from either embryonic or postnatal EGFP rat thymi were aggregated with donor cells obtained from back skin of wild type mouse or rat embryos (E13.5-E15.5) and implanted under the kidney capsule of athymic mice. **b)** Appearance of control skin aggregates with cultured HF stem cells, soon after transplantation (left panels) and 3 weeks later after the hair follicles have formed and incorporated $EGFP^+$ HF control cells (right panels). **c)** 3D confocal images showing the participation of $EGFP^+$ TECs (PRE16Cl11 and PRP1Cl11) to chimeric hair follicles and epidermis; keratin 14 immunostaining further demonstrating the participation of TECs to the outer root sheath (ORS) and epidermis (Epid.). Bars = 50 μm.



Supplementary Figure 3. Schematic representation showing the genealogy of clonal populations of TECs, sr1-TECs and sr2-TECs.

Supplementary Table 1. Summary of thymic aggregation assays

	Cell Type	Passage	N° of Growing Grafts	N° of GFP$^+$ Grafts	3D Thymic Differentiation K5/K14	MHCII	AIRE1	Other GFP$^+$ Structures
TECs	PRE16Clone11	V	1/1	1/1	+	+	nd	–
		VII	3/6	2/3	+	+	+	K5-K8 bilyer
		VIII	3/4	2/3	+	+	nd	–
	#65 PRE16Clone11*	III	2/4	2/2	+	+	+	subcapsular layer K5 Neg
	PRP1Bulk	III	2/3	1/2	+	+	nd	–
	PRP1Clone11	VII	3/6	1/3	+	+	+	–
	PRP7Clone10	VI	2/2	2/2	+	+	+	–
	#66 PRP7Clone10*	III	2/2	2/2	+	+	+	–
	PRP4moBulk	IV	0/2	–	–	–	–	–
	PRP4moClone04	V	3/3	1/3	+	+	+	–
	PRP4moClone09	VI	3/3	1/3	+	nd	nd	–
sr1-TECs	#217 PRE16Clone11	III	2/3	1/2	+	+	nd	Epid.-like, SG
		IV	3/4	2/3	+	+	+	–
	#217Scl02**	IV	3/5	3/3	+	+	+	–
	#218 PRE16Clone11	II	1/1	1/1	+	+	nd	–
		III	7/11	5/7	+	+	+	Epid.-like
		IV	2/3	2/2	+	+	+	–
	#218Scl06**	IV	3/4	2/3	+	nd	nd	–
	#57 PRP1Clone11	III	2/2	1/2	–	–	–	Enucleated cells
		IV	2/2	1/2	–	–	–	Enucleated cells
	#907 PRP4moBulk	V	3/4	2/3	+	+	nd	–
HF cells	YR219P5Bulk	IV	2/2	0/2	–	–	–	–
	YR219P5Clone07	XII	1/2	1/1	–	–	–	Enucleated cells
		XIII	4/5	1/4	–	–	–	Enucleated cells
	YR219P5Scl151	III	1/2	1/1	–	–	–	Enucleated cells
Other Epithelia	Footpad	III	3/3	1/3	–	–	–	Enucleated cells
	Oesophagus	IV	3/3	1/3	–	–	–	Enucleated cells
		V	2/2	1/2	–	–	–	Undifferentiated cells
	Vagina	IV	3/4	1/3	–	–	–	Undifferentiated cells
		V	2/2	1/2	–	–	–	Undifferentiated cells

*, TECs grafted into thymus and recovered back in culture before grafting again into thymus

**, sr1-TEC culture subcloned after recovering from long-term skin engraftment

SG, Sebaceous Gland

Epid, epiderm

nd, not determined

Neg, negative

Supplementary Table 1 - Summary of thymic aggregation assays. Thymic epithelial cells (TECs) were isolated from EGFP$^+$ rat thymi at different stages: embryonic day 16 (PRE16), postnatal day 1 (PRP1), postnatal day 7 (PRP7) and four and a half months (PRP4mo). Both bulk and clonal cultures were expanded for several passages (IV-VIII) *in vitro* and the progeny of these cells grafted into thymic aggregates under the kidney capsule of athymic mice. A fraction of growing grafts (15/24) incorporated EGFP$^+$ TECs that differentiated in 3D thymic structures with prevalent phenotype of medulla. Most of the cells differentiated *in vivo* and expressed MHC-class II antigens while a consistent subpopulation expressed also the nuclear protein AIRE. TECs recovered from the 1st skin transplantation (sr1-TECs – #217, #218 and #907) also incorporated into thymic structures, while sr1-TEC #57 never integrated. rs1-TEC #217 gave rise also to epidermal-like structures and sebaceous gland in some thymic grafts. rs1-TEC #217 and rs1-TEC #218 were subcloned and the progeny of these cells incorporated into the thymic structures and expressed marker of thymic functionality. Hair follicle cultivated cells (HF cells – YR219P5) never integrated into thymus, however EGFP$^+$ cyst –like structures constituted of enucleated cells could be observed. Similarly, EGFP$^+$ cells derived from other stratified epithelia (footpad, esophagus and vagina) never integrated into the thymic 3D network.

Supplementary Table 2. Summary of skin reconstitution assays

	Cell Type	Passage	N° of GFP$^+$ grafts	Long Term Incorporation	GFP$^+$ Structures Epid.	HF	SG	Incorporation Efficiency
TECs	PRE16Clone11	VI	2/6	2/2	+	+	+	33% LT
		VIII	2/2	2/2	+	+	+	100% LT
	PRP1Bulk	II	0/6	–	–	–	–	0%
	PRP1Clone11	VI	2/8	1/2	–	Infund.	+	12.5% ST, 12.5% LT
		VII	1/3	1/1	–	+	+	33%, LT
	PRP1Clone07b	V	0/5	–	–	–	–	0%
	PRP1Clone09b	V	0/2	–	–	–	–	0%
	PRP7Bulk	II	1/3	0/1	–	–	+	10% ST
	PRP7Clone10	IV	1/7	0/1	–	–	+	14% ST
		V	2/3	1/2	–	+	+	33% ST, 33% LT
	PRP4moBulk	IV	3/6	2/3	–	+	Cyst	15% ST, 30% LT
	PRP4moClone04	V	1/3	1/1	–	–	+	33%, LT
	PRP4moClone09	VI	0/2	–	–	–	–	0%
sr1-TECs	#218 PRE16Clone11	III	2/2	2/2	–	+	+	100% LT
		IV	3/3	3/3	+	+	+	100% LT
	#218 Scl06**	III	2/2	2/2	+	+	+	100% LT
		IV	2/2	2/2	+	+	+	100% LT
	#217 PRE16Clone11	II	2/2	2/2	+	+	+	100% LT
		III	3/3	3/3	–	+	+	100% LT
	#217 Scl02**	III	1/2	1/1	+	+	+	100% LT
		IV	2/2	2/2	+	+	+	100% LT
	#57 PRP1Clone11	III	2/2	2/2	+	+	+	100% LT
	#907 PRP4moBulk	V	4/4	4/4	+	+	+	100% LT
HF cells	YR219P5Clone07	XII	6/6	6/6	–	+	+	100% LT
	YR219P5Cl07Scl151	III	1/1	1/1	–	+	+	100% LT

**, sr1-TEC culture subcloned after recovering from long-term skin engraftment
Epid, Epidermis
HF, Hair Follicle
SG, Sebaceous Gland
Infund, Infundibulum
LT, Long Term
ST, Short Term

Supplementary Table 2 - Summary of skin reconstitution assays. Thymic epithelial cells (TECs) were isolated from EGFP$^+$ rat thymi at different stages: embryonic day 16 (PRE16), postnatal day 1 (PRP1), postnatal day 7 (PRP7) and four and a half months (PRP4mo). Both bulk and clonal cultures were expanded for several passages (II-VIII) *in vitro* and the progeny of these cells grafted into skin reconstitution assay. 15 out of 56 grafts incorporated EGFP$^+$ TECs that differentiated into epidermis, sebaceous gland and hair follicle structures. A fraction of EGFP$^+$ positive grafts (10/15) incorporated EGFP$^+$ TECs at long term (> 60 days) into cycling hair follicles, sebaceous gland and also epidermis. In each graft 1-30 cycling hair follicles and differentially extended areas of epidermis were found. TECs recovered from the 1st skin transplantation (sr1-TECs – #217, #218, #57 and #907) also incorporated into all functional skin structures, but in sharp contrast with the 1st transplantation incorporated with an efficiency of 100%. In each of the serial transplants hundreds of hair follicles and extended areas of epidermis were formed. rs1-TEC #217 and rs1-TEC #218 were subcloned and the progeny of these cells incorporated into all skin structures. HF cells (YR219P5) always incorporated into skin forming hair follicles and sebaceous gland at long term, but never formed epidermis.

Supplementary Table 3. List of genes up- and down- regulated in TECs after the 1st and the 2nd skin transplantation

ID	Gene.Symbol	Gene.Description	sr1-TECs vs TECs	sr2-TECs vs TECs	HF cells vs TECs
10705654	Lgals7	lectin, galactose binding, soluble 7	35.44	39.73	72.20
10717240	Moxd1	monooxygenase, DBH-like 1	8.85	14.62	21.32
10823949	Lrat	lecithin-retinol acyltransferase (phosphatidylcholine-retinol-O	1.87	3.02	18.27
10874811	C1qdc2	C1q domain containing 2	3.25	3.33	14.38
10752295	Tbx1	T-box 1	2.83	2.76	13.25
10859392	Mgst1	microsomal glutathione S-transferase 1	4.73	2.86	10.76
10725778	Nupr1	nuclear protein 1	6.45	9.49	10.18
10723576	Prss23	protease, serine, 23	2.85	3.36	9.77
10867026	Bhlhb3	basic helix-loop-helix domain containing, class B3	1.37	1.52	8.45
10834098	Il1f5	interleukin 1 family, member 5 (delta)	2.18	3.49	8.44
10922096	Tcfap2b	transcription factor AP-2 beta	1.82	1.91	8.35
10756665	Ocm	oncomodulin	10.71	28.65	8.08
10892835	Itgb8	integrin beta 8	2.46	3.74	7.38
10826371	Palmd	palmdelphin	-1.31	1.39	6.32
10794599	Nedd9	neural precursor cell expressed, developmentally down-regul	4.07	4.12	6.07
10754592	RGD1564641	similar to Neurogenic locus notch homolog protein 2 precurso	1.31	1.60	6.01
10864874	Rassf4	Ras association (RalGDS/AF-6) domain family member 4	1.28	1.79	5.94
10911818	Cd109	CD109 molecule	4.42	5.80	5.87
10781378	Hr	hairless	2.54	3.05	5.85
10876896	Ctnnal1	catenin (cadherin associated protein), alpha-like 1	2.38	3.13	5.84
10875287	Rdhe2	retinal short chain dehydrogenase reductase 2	1.20	1.39	5.67
10759513	Lass4	LAG1 homolog, ceramide synthase 4	2.35	3.91	5.67
10747084	Krt10	keratin 10	1.69	3.49	5.59
10863215	Vamp5	vesicle-associated membrane protein 5	1.60	2.24	5.28
10846916	---	---	3.51	6.37	4.91
10891303	Tgfb3	transforming growth factor, beta 3	1.26	1.27	4.74
10796090	Calml3	calmodulin-like 3	5.12	5.29	4.68
10745438	Slc6a4	solute carrier family 6 (neurotransmitter transporter, serotoni	1.83	2.20	4.58
10907815	Casp1	caspase 1	7.30	5.38	4.56
10822234	Car2	carbonic anhydrase II	1.77	1.43	4.36
10706059	Sbsn	suprabasin	1.81	2.02	4.35
10749975	---	---	1.27	1.40	4.27
10838354	Lgr4	leucine-rich repeat-containing G protein-coupled receptor 4	1.09	1.53	4.18
10791935	Zdhhc2	zinc finger, DHHC domain containing 2	2.47	2.45	4.13
10797138	Tgfbi	transforming growth factor, beta induced	3.01	3.78	4.12
10771919	Sult1d1	sulfotransferase family 1D, member 1	10.94	11.36	4.10
10702250	Enpp1	ectonucleotide pyrophosphatase/phosphodiesterase 1	5.74	5.24	4.04
10830624	Cd24	CD24 molecule	2.47	2.33	4.04
10896661	Fam83a	family with sequence similarity 83, member A	1.44	2.41	3.97
10706895	Fut1	fucosyltransferase 1	3.41	3.84	3.86
10701934	Il22ra2	interleukin 22 receptor, alpha 2	3.08	2.17	3.73
10796220	Gata3	GATA binding protein 3	3.23	3.53	3.71
10922816	Il1r2	interleukin 1 receptor, type II	3.41	3.23	3.71
10834109	Il1rn	interleukin 1 receptor antagonist	2.47	2.86	3.69
10839144	Ckmt1	creatine kinase, mitochondrial 1, ubiquitous	3.78	5.35	3.66
10809538	Capns2	calpain, small subunit 2	1.83	2.15	3.64
10779832	Rnase4	ribonuclease, RNase A family 4	2.34	2.36	3.63
10763421	Dsel	dermatan sulfate epimerase-like	6.27	5.21	3.50
10859671	LOC362464	similar to aryl hydrocarbon receptor nuclear translocator-like	1.33	1.57	3.45
10876730	RGD1307218	similar to RIKEN cDNA 2810432L12	1.35	1.55	3.42
10717233	Ctgf	connective tissue growth factor	3.21	2.57	3.42
10758351	Gpr109a	G protein-coupled receptor 109A	1.71	3.43	3.41
10808377	Crispld2	cysteine-rich secretory protein LCCL domain containing 2	2.18	2.24	3.41
10706065	Dmkn	dermokine	1.89	2.53	3.40
10720884	Fxyd3	FXYD domain-containing ion transport regulator 3	2.10	2.22	3.39
10730349	Scd1	stearoyl-Coenzyme A desaturase 1	3.32	4.13	3.38
10774596	Bcl11a	B-cell CLL/lymphoma 11A (zinc finger protein)	1.26	1.33	3.38
10891240	Pgf	placental growth factor	2.02	2.28	3.37
10725782	Sult1a1	sulfotransferase family 1A, phenol-preferring, member 1	2.57	1.97	3.30
10835958	Lhx2	LIM homeobox protein 2	2.00	2.03	3.29
10767723	Atp2b4	ATPase, Ca++ transporting, plasma membrane 4	1.32	1.38	3.20
10706412	Klk10	kallikrein related-peptidase 10	3.23	4.34	3.19
10888620	Clip4	CAP-GLY domain containing linker protein family, member 4	3.05	3.10	3.19
10790581	LOC290595	hypothetical gene supported by AF152002	14.42	29.74	3.18
10801343	Sh3rf2	SH3 domain containing ring finger 2	3.48	5.55	3.17
10793243	Irx1	Iroquois related homeobox 1 (Drosophila)	2.00	1.81	3.16
10719530	Apoe	apolipoprotein E	2.88	2.61	3.10
10816153	Tmem154	transmembrane protein 154	1.93	2.20	3.09
10801434	Spink5	serine peptidase inhibitor, Kazal type 5	1.59	1.64	3.08

10739357	Sox9	SRY-box containing gene 9	1.42	1.78	3.05
10821824	Slc1a3	solute carrier family 1 (glial high affinity glutamate transporte	1.50	1.26	2.98
10863213	---	---	1.34	1.64	2.97
10798119	Nqo2	NAD(P)H dehydrogenase, quinone 2	-1.21	-1.83	2.94
10794200	Msx2	msh homeobox 2	1.35	1.73	2.93
10793981	Pitx1	paired-like homeodomain transcription factor 1	2.08	3.81	2.93
10817527	LOC690102	similar to histone H2A	1.46	2.01	2.93
10912245	Plscr4	phospholipid scramblase 4	2.11	1.61	2.92
10729314	Gda	guanine deaminase	1.80	1.13	2.92
10889027	RGD1309228	similar to putative protein, with at least 9 transmembrane do	1.44	1.07	2.92
10708531	Rab38	Rab38, member of RAS oncogene family	1.83	1.86	2.92
10770759	Sertad4	SERTA domain containing 4	1.72	2.25	2.92
10796987	---	---	1.93	2.50	2.90
10788204	Snx25	sorting nexin 25	1.48	1.78	2.88
10784346	Sgcg	sarcoglycan, gamma (dystrophin-associated glycoprotein)	1.25	1.11	2.86
10855449	Gpnmb	glycoprotein (transmembrane) nmb	1.88	2.24	2.85
10903674	Trps1	trichorhinophalangeal syndrome I (human)	1.21	1.50	2.82
10796440	Pter	phosphotriesterase related	1.51	1.69	2.81
10917215	Bco2	beta-carotene oxygenase 2	1.71	2.25	2.81
10791650	---	---	1.54	-1.07	2.80
10735424	Fam64a	family with sequence similarity 64, member A	1.40	1.56	2.80
10808274	Cdh13	cadherin 13	1.94	3.65	2.79
10925365	Ramp1	receptor (calcitonin) activity modifying protein 1	3.41	2.34	2.77
10730322	RGD1310475	similar to RIKEN cDNA 0610010D20	1.53	1.44	2.75
10809216	Gpr56	G protein-coupled receptor 56	2.54	2.49	2.74
10719824	Cnfn	cornifelin	2.04	2.56	2.74
10832175	Cbs	cystathionine beta synthase	1.74	1.86	2.74
10867955	Epha7	Eph receptor A7	-1.15	-1.26	2.73
10911145	Car12	carbonic anyhydrase 12	2.78	2.94	2.73
10756487	Cdx2	caudal type homeo box 2	2.71	4.57	2.73
10749977	---	---	1.22	1.35	2.73
10877630	Megf9	multiple EGF-like-domains 9	1.85	2.12	2.71
10738972	Mrc2	mannose receptor, C type 2	1.66	1.71	2.71
10822107	Fam105a	family with sequence similarity 105, member A	1.93	3.54	2.71
10817068	S100a7a	S100 calcium binding protein A7A	3.74	2.77	2.69
10924427	Wnt10a	wingless related MMTV integration site 10a	2.92	2.43	2.68
10702598	Ppp1r14c	protein phosphatase 1, regulatory (inhibitor) subunit 14c	2.05	2.00	2.68
10887802	Cdca7l	cell division cycle associated 7 like	1.62	1.61	2.66
10804292	RGD1563060	similar to AVLV472	2.06	1.88	2.66
10855008	Gstk1	glutathione S-transferase kappa 1	1.69	1.23	2.66
10906736	Vdr	vitamin D receptor	2.44	3.16	2.66
10888610	LOC683626	similar to limb-bud and heart	3.50	2.52	2.65
10796989	---	---	2.10	2.43	2.65
10912161	Ctsh	cathepsin H	2.84	3.06	2.65
10771830	Adamts3	ADAM metallopeptidase with thrombospondin type 1, motif 3	1.14	1.33	2.64
10802324	Sec11c	SEC11 homolog C (S. cerevisiae)	1.31	1.39	2.63
10705411	---	---	2.18	2.61	2.61
10774605	---	---	1.07	1.10	2.61
10771826	Adamts3	ADAM metallopeptidase with thrombospondin type 1, motif 3	1.23	1.35	2.61
10789442	Gas6	growth arrest specific 6	-1.03	-1.21	2.60
10843424	Dpp7	dipeptidylpeptidase 7	1.36	1.38	2.60
10895773	Lrig3	leucine-rich repeats and immunoglobulin-like domains 3	1.96	2.18	2.55
10860457	Sema3e	sema domain, immunoglobulin domain (Ig), short basic doma	1.24	1.21	2.55
10813969	Ank	progressive ankylosis	1.02	-1.22	2.54
10759852	RGD1307034	similar to hypothetical protein CG003	1.48	2.04	2.51
10716667	Ust	uronyl-2-sulfotransferase	-1.01	-1.04	2.51
10765534	Adamts4	a disintegrin-like and metallopeptidase (reprolysin type) with	-1.03	1.05	2.51
10798459	Hist1h2bb	histone cluster 1, H2bb	1.37	1.57	2.50
10903676	Trps1	trichorhinophalangeal syndrome I (human)	1.08	1.31	2.49
10765335	Creg1	cellular repressor of E1A-stimulated genes 1	2.21	2.44	2.49
10707790	Pcsk6	proprotein convertase subtilisin/kexin type 6	1.57	2.09	2.48
10832920	Ddit4	DNA-damage-inducible transcript 4	2.42	2.00	2.47
10937734	Pir	pirin	2.41	2.65	2.46
10891133	Abcd4	ATP-binding cassette, sub-family D (ALD), member 4	2.06	2.64	2.44
10888608	LOC683626	similar to limb-bud and heart	3.13	2.45	2.44
10790563	Pcdh21	protocadherin 21	1.51	1.56	2.43
10799914	LOC680565	hypothetical protein LOC680565	1.33	1.60	2.43
10918066	Glce	glucuronyl C5-epimerase	1.54	1.61	2.43
10911001	Rbpms2	RNA binding protein with multiple splicing 2	1.14	1.26	2.41
10771258	Slc10a6	solute carrier family 10 (sodium/bile acid cotransporter family	1.74	1.97	2.41
10708695	Odz4	odd Oz/ten-m homolog 4 (Drosophila)	1.36	1.63	2.40
10796845	Irx2	Iroquois related homeobox 2 (Drosophila)	1.59	1.60	2.40

10850525	Thbd	thrombomodulin	2.07	2.49	2.39
10825925	Gstm1	glutathione S-transferase, mu 1	3.52	5.28	2.38
10786108	Rarb	retinoic acid receptor, beta	1.27	1.38	2.36
10782166	Pcca	propionyl-coenzyme A carboxylase, alpha polypeptide	1.90	1.60	2.36
10933984	Pcyt1b	phosphate cytidylyltransferase 1, choline, beta isoform	1.10	1.36	2.36
10892939	Prim1	DNA primase, p49 subunit	1.26	1.26	2.36
10767631	Nfasc	neurofascin	-1.01	-1.21	2.35
10864048	Nup210	nucleoporin 210	2.13	2.15	2.34
10827454	Acadm	acyl-Coenzyme A dehydrogenase, medium chain	1.41	1.25	2.34
10867389	Rgs20	regulator of G-protein signaling 20	1.46	1.81	2.34
10924441	---	---	1.60	1.17	2.33
10901409	Chst11	carbohydrate sulfotransferase 11	-1.27	-1.13	2.33
10811347	Plcg2	phospholipase C, gamma 2	1.48	1.63	2.32
10887774	Rapgef5	Rap guanine nucleotide exchange factor (GEF) 5	1.88	2.18	2.31
10862634	Scrn1	secernin 1	-1.38	-1.27	2.31
10722818	Slco3a1	solute carrier organic anion transporter family, member 3a1	-1.03	1.08	2.31
10797161	RGD1563703	similar to hypothetical protein E230025K15	2.14	2.75	2.31
10937362	Pak3	p21 (CDKN1A)-activated kinase 3	1.48	1.12	2.30
10878705	Cdkn2c	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	2.01	1.65	2.29
10825890	Gstm3	glutathione S-transferase, mu type 3	1.70	1.87	2.29
10785826	Abcc4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	1.78	2.06	2.28
10840650	LOC366222	similar to transcription factor RAM2	1.29	1.38	2.27
10701902	RGD1308124	similar to KIAA1357 protein	1.67	2.27	2.27
10777788	Spon2	spondin 2, extracellular matrix protein	-1.47	-1.12	2.26
10763820	RGD1562617	similar to RAB7-like protein	2.04	2.43	2.26
10830189	Gja1	gap junction protein, alpha 1	1.44	1.85	2.26
10866970	Pthlh	parathyroid hormone-like peptide	1.69	2.05	2.25
10717779	Fbxo5	F-box protein 5	2.01	1.70	2.25
10798921	---	---	1.94	2.19	2.25
10707597	Atp10a	ATPase, class V, type 10A	-1.12	-1.02	2.24
10874048	RGD1563533	similar to novel protein	2.96	3.00	2.24
10745533	Accn1	amiloride-sensitive cation channel 1, neuronal (degenerin)	1.12	1.39	2.23
10795260	---	---	1.03	1.15	2.23
10740891	Paqr4	progestin and adipoQ receptor family member IV	1.94	1.87	2.23
10795989	Pfkp	phosphofructokinase, platelet	1.26	1.62	2.23
10797660	Aspn	asporin	1.44	2.09	2.22
10827517	Cth	cystathionase (cystathionine gamma-lyase)	1.73	1.51	2.22
10853995	Asb15	ankyrin repeat and SOCS box-containing protein 15	-1.01	-1.06	2.21
10712104	RGD1309350	similar to transthyretin (4L369)	2.76	2.79	2.21
10869303	Slc31a2	solute carrier family 31, member 2	1.29	1.18	2.21
10868853	Foxe1	forkhead box E1 (thyroid transcription factor 2)	1.11	1.13	2.21
10901083	Znf124	zinc finger protein 124 (HZF-16)	1.31	1.19	2.21
10746899	---	---	1.24	1.13	2.21
10857435	Mitf	microphthalmia-associated transcription factor	1.48	1.73	2.21
10717311	RGD1306962	similar to dJ55C23.6 gene product	2.24	2.16	2.20
10832482	Ndg2	Nur77 downstream gene 2	2.38	1.86	2.20
10835355	Ass1	argininosuccinate synthetase 1	1.32	1.82	2.19
10908391	Slc44a2	solute carrier family 44, member 2	1.93	1.99	2.19
10707756	Tarsl2	threonyl-tRNA synthetase-like 2	1.12	1.21	2.19
10918569	Aqp9	aquaporin 9	2.29	3.00	2.19
10886269	Flrt2	fibronectin leucine rich transmembrane protein 2	-1.02	1.27	2.18
10714106	Fam111a	family with sequence similarity 111, member A	1.10	1.30	2.18
10889213	Vsnl1	visinin-like 1	1.45	2.01	2.18
10754218	Fstl1	follistatin-like 1	-1.04	-1.27	2.18
10798507	LOC680615	similar to histone 2a	1.10	1.17	2.17
10854199	Tspan33	tetraspanin 33	3.19	2.81	2.17
10732644	Stc2	stanniocalcin 2	3.80	3.12	2.16
10742555	Tcf7	transcription factor 7, T-cell specific	1.32	1.54	2.15
10910191	---	---	1.51	1.77	2.15
10797786	Gmpr	guanosine monophosphate reductase	1.46	1.89	2.15
10706404	Klk11	kallikrein related-peptidase 11	2.05	2.91	2.14
10864176	Prickle2	prickle-like 2 (Drosophila)	1.33	2.01	2.14
10848416	Fsip1	fibrous sheath interacting protein 1	1.30	1.24	2.14
10939516	Tsc22d3	TSC22 domain family 3	2.86	2.28	2.14
10745342	Evi2a	ecotropic viral integration site 2A	-1.13	-1.15	2.13
10818291	Gstm5	glutathione S-transferase, mu 5	1.75	1.39	2.13
10735012	Sox15	SRY-box containing gene 15	1.90	2.39	2.13
10914935	RGD1310444	LOC363015	1.46	1.54	2.12
10750329	Cbr3	carbonyl reductase 3	1.93	1.83	2.12
10812922	Elovl7	ELOVL family member 7, elongation of long chain fatty acids	1.72	2.19	2.12
10800342	Dsg1b	desmoglein 1 beta	1.56	3.19	2.11
10892265	Ckb	creatine kinase, brain	1.23	1.15	2.11

10836638	Klhl23	kelch-like 23 (Drosophila)	1.81	2.00	2.11
10840076	Prnp	prion protein	2.40	2.58	2.10
10809563	Irx5	iroquois homeobox 5	1.54	1.70	2.10
10755013	Il1rap	interleukin 1 receptor accessory protein	1.21	1.35	2.09
10881766	Rbp7	retinol binding protein 7, cellular	1.50	1.47	2.09
10917112	Nnmt	nicotinamide N-methyltransferase	1.25	1.73	2.09
10908369	Slc44a2	solute carrier family 44, member 2	2.03	2.42	2.09
10801199	Pcdhb17	protocadherin beta 17	1.48	1.71	2.08
10726702	RGD1560565	similar to Tumor protein p53 inducible protein 5	1.78	1.99	2.08
10785846	Abcc4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	1.62	1.80	2.08
10847932	Depdc7	DEP domain containing 7	1.50	1.34	2.08
10880583	Clic4	chloride intracellular channel 4 (mitochondrial)	1.40	1.61	2.07
10712548	Slc22a18	solute carrier family 22 (organic cation transporter), member	1.76	1.57	2.07
10859738	Ccdc91	coiled-coil domain containing 91	1.45	1.82	2.07
10886387	RGD1311756	similar to hypothetical protein FLJ20950	1.65	1.91	2.07
10803323	Cdh2	cadherin 2	-1.44	1.27	2.07
10794589	RGD1305679	similar to 9530008L14Rik protein	3.01	3.70	2.07
10726280	Oat	ornithine aminotransferase	1.52	1.45	2.06
10863471	Dok1	docking protein 1	1.16	1.16	2.06
10722549	Klf13	Kruppel-like factor 13	2.03	2.07	2.06
10832076	LOC689755	hypothetical protein LOC689755	-1.02	1.12	2.05
10733730	Galnt10	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylga	1.38	1.45	2.05
10847806	Ehf	ets homologous factor	2.07	3.17	2.05
10897360	Gpt	glutamic-pyruvate transaminase (alanine aminotransferase)	2.45	2.01	2.05
10826002	Celsr2	cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo h	1.96	2.19	2.05
10931147	Steap3	STEAP family member 3	1.53	1.76	2.05
10813353	---	---	1.56	1.50	2.04
10797705	Ninj1	ninjurin 1	1.12	-1.01	2.04
10730677	Obfc1	oligonucleotide/oligosaccharide-binding fold containing 1	1.93	1.48	2.02
10779835	Ang1	angiogenin, ribonuclease A family, member 1	2.02	1.98	2.02
10737949	Ppp1r1b	protein phosphatase 1, regulatory (inhibitor) subunit 1B	1.26	1.22	2.01
10715149	Tmem20	transmembrane protein 20	1.26	1.82	2.01
10918770	Fam83b	family with sequence similarity 83, member B	1.29	1.81	2.01
10897574	Gcat	glycine C-acetyltransferase (2-amino-3-ketobutyrate-coenzyn	1.53	1.21	2.00
10771771	Rassf6	Ras association (RalGDS/AF-6) domain family member 6	2.68	2.26	2.00
10829791	Zfp365	zinc finger protein 365	-1.19	-1.02	2.00
10858655	C1r	complement component 1, r subcomponent	2.68	4.00	1.99
10791677	Acsl1	acyl-CoA synthetase long-chain family member 1	2.13	2.65	1.98
10754580	Muc13	mucin 13, epithelial transmembrane	1.77	3.02	1.97
10797527	Gadd45g	growth arrest and DNA-damage-inducible 45 gamma	2.99	2.05	1.96
10752081	Ehhadh	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A del	1.72	2.02	1.96
10912088	Nt5e	5' nucleotidase, ecto	2.70	2.14	1.96
10763850	Pm20d1	peptidase M20 domain containing 1	1.53	2.02	1.96
10830561	Sesn1	sestrin 1	2.08	1.73	1.95
10840020	Cdc25b	cell division cycle 25 homolog B (S. pombe)	2.23	2.43	1.95
10891850	Itpk1	inositol 1,3,4-triphosphate 5/6 kinase	1.84	2.33	1.95
10825575	Olfml3	olfactomedin-like 3	-1.62	-2.55	-1.94
10741189	Slc9a3r2	solute carrier family 9 (sodium/hydrogen exchanger), membe	-1.46	-2.02	-1.94
10804164	Prelid2	PRELI domain containing 2	-3.37	-3.05	-1.96
10798943	Snurf	SNRPN upstream reading frame	-5.74	-5.94	-1.96
10889263	Trib2	tribbles homolog 2 (Drosophila)	-2.05	-2.34	-1.97
10877992	---	---	-3.66	-3.48	-1.98
10791565	Vegfc	vascular endothelial growth factor C	-1.90	-2.11	-1.99
10902313	Glipr1	GLI pathogenesis-related 1 (glioma)	2.69	2.20	-2.00
10907324	LOC683264	similar to Polypeptide N-acetylgalactosaminyltransferase 6 (P	-1.02	1.01	-2.00
10794031	Dbn1	drebrin 1	-2.65	-3.04	-2.00
10853401	Steap2	six transmembrane epithelial antigen of the prostate 2	2.22	2.08	-2.01
10714616	Slc1a1	solute carrier family 1 (neuronal/epithelial high affinity glutar	-1.60	-1.91	-2.01
10850070	Rnf24	ring finger protein 24	-1.21	-1.25	-2.01
10734291	Adora2b	adenosine A2B receptor	-1.77	-2.15	-2.01
10831595	Psmb8	proteasome (prosome, macropain) subunit, beta type 8 (larg	-1.43	-1.56	-2.02
10797648	Ogn	osteoglycin	-1.81	-1.52	-2.02
10824611	Slc27a3	solute carrier family 27 (fatty acid transporter), member 3	-1.88	-1.74	-2.02
10740018	LOC688296	similar to chromosome 17 open reading frame 27	-1.15	-1.16	-2.02
10871229	Pik3r3	phosphatidylinositol 3 kinase, regulatory subunit, polypeptide	-1.87	-1.99	-2.03
10827835	RT1-T24-1	histocompatibility 2, T region locus 24	1.14	1.13	-2.03
10831099	RT1-CE5	RT1 class I, CE5	-1.11	-1.57	-2.03
10878016	Elavl2	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (	-2.04	-2.54	-2.03
10775628	Anxa3	annexin A3	-1.39	-1.30	-2.03
10889590	Prkar2b	protein kinase, cAMP dependent regulatory, type II beta	1.16	1.13	-2.04
10719665	RGD1565787	similar to RIKEN cDNA 1810065E05	-2.17	-2.50	-2.05
10803947	Hbegf	heparin-binding EGF-like growth factor	-1.63	-1.73	-2.06

10820577	F2rl1	coagulation factor II (thrombin) receptor-like 1	-1.45	-1.40	-2.06
10788881	LOC684785	similar to pleckstrin homology domain-containing, family A (p	-1.29	-1.21	-2.06
10771535	LOC305633	similar to Antxr2 protein	-1.12	1.03	-2.06
10804319	RGD1561988	similar to Colorectal mutant cancer protein (MCC protein)	-1.47	-1.98	-2.06
10884158	RGD1560812	RGD1560812	-1.77	-1.85	-2.06
10881211	Efhd2	EF hand domain containing 2	-1.88	-1.89	-2.06
10822043	Cdh6	cadherin 6	-1.93	-2.18	-2.06
10767465	Il24	interleukin 24	-1.88	-2.12	-2.07
10760971	Pcolce	procollagen C-endopeptidase enhancer protein	-1.41	-2.34	-2.07
10811278	Maf	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (	-1.64	-1.70	-2.07
10788312	LOC685630	hypothetical protein LOC685630	-1.06	-1.28	-2.07
10722489	Snrpn	small nuclear ribonucleoprotein N	-5.26	-5.18	-2.07
10823216	RGD1562629	similar to neurobeachin	-1.50	-1.98	-2.07
10801778	---	---	-1.00	1.14	-2.07
10704578	MGC72567	similar to coiled-coil domain containing 8	-3.05	-4.61	-2.08
10866459	RGD1308734	similar to RIKEN cDNA 1100001H23	1.27	1.21	-2.08
10934038	Gspt2	G1 to S phase transition 2	-1.58	-2.19	-2.08
10884356	---	---	-2.56	-3.27	-2.09
10786851	Arhgap22	Rho GTPase activating protein 22	-1.86	-2.23	-2.09
10721667	Fcgrt	Fc receptor, IgG, alpha chain transporter	-1.37	-1.62	-2.10
10862415	Tmem176b	transmembrane protein 176B	-1.62	-2.03	-2.10
10870342	Pde4b	phosphodiesterase 4B, cAMP specific	-2.13	-2.21	-2.10
10879928	Gja4	gap junction protein, alpha 4	-1.30	-1.54	-2.10
10818823	Pde5a	phosphodiesterase 5A, cGMP-specific	1.10	1.09	-2.10
10702829	Synj2	synaptojanin 2	-1.20	-1.09	-2.11
10880994	Padi1	peptidyl arginine deiminase, type I	-1.52	-1.80	-2.11
10756229	Insr	insulin receptor	-1.10	-1.15	-2.11
10838056	Trp53i11	transformation related protein 53 inducible protein 11	1.40	1.19	-2.11
10924853	Itm2c	integral membrane protein 2C	-1.24	-1.46	-2.12
10843331	RGD1308019	similar to hypothetical protein FLJ20245	-1.52	-1.47	-2.13
10706418	Klk9	kallikrein related-peptidase 9	-1.06	-1.08	-2.13
10756251	---	---	-1.13	-1.21	-2.13
10772030	RGD1306446	similar to RIKEN cDNA 9930032O22 gene	1.63	1.43	-2.14
10846220	Chn1	chimerin (chimaerin) 1	-1.74	-2.01	-2.14
10782271	Plau	plasminogen activator, urokinase	-1.83	-2.46	-2.14
10765096	Tnfsf18	tumor necrosis factor (ligand) superfamily, member 18	-2.68	-2.33	-2.14
10719922	Cyp2s1	cytochrome P450, family 2, subfamily s, polypeptide 1	1.40	1.14	-2.15
10865329	Apobec1	apolipoprotein B editing complex 1	1.87	1.30	-2.15
10824165	Pear1	platelet endothelial aggregation receptor 1	-2.63	-3.19	-2.15
10744370	Slc16a13	solute carrier family 16 (monocarboxylic acid transporters), n	-1.74	-1.67	-2.16
10902249	Nav3	neuron navigator 3	-1.36	-1.38	-2.16
10771655	Cxcl10	chemokine (C-X-C motif) ligand 10	-1.57	-1.64	-2.16
10891910	isg12(b)	putative ISG12(b) protein	1.27	1.16	-2.16
10864441	Rybp	RING1 and YY1 binding protein	-1.59	-1.63	-2.16
10725279	Gprc5b	G protein-coupled receptor, family C, group 5, member B	-2.26	-2.26	-2.17
10884215	Twist1	twist gene homolog 1 (Drosophila)	-1.83	-2.32	-2.17
10759096	Trpv4	transient receptor potential cation channel, subfamily V, mem	-1.52	-1.46	-2.17
10800471	Dtna	dystrobrevin alpha	-2.97	-3.07	-2.17
10772791	LOC498368	similar to RIKEN cDNA 0610040J01	-1.18	-1.43	-2.18
10740016	LOC688296	similar to chromosome 17 open reading frame 27	-1.25	-1.26	-2.18
10751018	Abhd10	abhydrolase domain containing 10	-1.41	-1.34	-2.18
10763367	Serpinb2	serine (or cysteine) peptidase inhibitor, clade B, member 2	-1.29	-1.15	-2.18
10803977	---	---	-1.69	-1.76	-2.18
10708281	Pde8a	phosphodiesterase 8A	-1.71	-1.83	-2.18
10739994	RGD1308168	similar to chromosome 17 open reading frame 27	-1.35	-1.29	-2.19
10743914	Chd3	chromodomain helicase DNA binding protein 3	-1.61	-1.62	-2.19
10794866	Serpinb1a	serine (or cysteine) proteinase inhibitor, clade B, member 1a	-1.06	-1.10	-2.19
10848486	RGD1565536	similar to hypothetical protein	-1.89	-2.13	-2.19
10760559	RGD1560686	similar to sidekick 1	-1.76	-1.93	-2.21
10752576	Pkp2	plakophilin 2	-1.38	-1.50	-2.21
10719432	Fosb	FBJ osteosarcoma oncogene B	-1.09	-1.24	-2.22
10925405	Klhl30	kelch-like 30 (Drosophila)	-1.06	-1.48	-2.22
10840555	Pax1	paired box gene 1	-2.32	-2.47	-2.23
10832197	Snf1lk	SNF1-like kinase	-1.45	-1.89	-2.24
10887679	Vipr2	vasoactive intestinal peptide receptor 2	-1.61	-1.43	-2.25
10749874	Gbe1	glucan (1,4-alpha-), branching enzyme 1	-1.79	-1.66	-2.25
10770082	Ifi204	interferon activated gene 204	-1.39	-1.24	-2.26
10729795	Slc16a12	solute carrier family 16 (monocarboxylic acid transporters), n	1.16	-1.09	-2.27
10741851	---	---	-1.28	-1.44	-2.27
10821632	Ghr	growth hormone receptor	-1.45	-1.46	-2.27
10724391	RGD1563970	similar to Tripartite motif protein 30-like	-2.10	-2.25	-2.28
10894939	RGD1565947	similar to netrin 4	1.48	1.49	-2.28

10782533	Slc4a7	solute carrier family 4, sodium bicarbonate cotransporter, me	-1.01	-1.00	-2.28
10746483	Itga3	integrin alpha 3	-1.49	-1.41	-2.28
10748200	Limd2	LIM domain containing 2	-1.48	-2.10	-2.28
10873021	Wnt4	wingless-related MMTV integration site 4	-1.51	-1.62	-2.29
10758724	LOC690517	similar to Ornithine decarboxylase (ODC)	-2.32	-2.35	-2.30
10817105	---	---	-1.12	-1.27	-2.30
10775519	Agpat9	1-acylglycerol-3-phosphate O-acyltransferase 9	-1.52	-1.66	-2.30
10820052	Rhobtb3	Rho-related BTB domain containing 3	-1.40	-1.89	-2.31
10925415	Fam132b	family with sequence similarity 132, member B	1.22	-1.17	-2.31
10807576	Has3	hyaluronan synthase 3	-2.43	-2.11	-2.32
10903816	Sntb1	syntrophin, basic 1	-1.01	-1.03	-2.32
10751469	Parp14	poly (ADP-ribose) polymerase family, member 14	-2.00	-2.06	-2.33
10861890	Creb3l2	cAMP responsive element binding protein 3-like 2	-1.32	-1.48	-2.33
10739984	RGD1308168	similar to chromosome 17 open reading frame 27	-1.22	-1.24	-2.34
10923036	Gulp1	GULP, engulfment adaptor PTB domain containing 1	-1.96	-2.70	-2.34
10824655	Npr1	natriuretic peptide receptor 1	-1.71	-1.57	-2.35
10833180	Srgn	serglycin	-1.68	-2.11	-2.35
10767577	Pctk3	PCTAIRE-motif protein kinase 3	-1.72	-1.83	-2.35
10774171	Upp1	uridine phosphorylase 1	-2.73	-3.34	-2.36
10716080	Dusp5	dual specificity phosphatase 5	-1.83	-2.26	-2.36
10909141	Olr1219	olfactory receptor 1219	-1.89	-1.75	-2.36
10880163	RGD1561205	similar to RIKEN cDNA 2610200G18	-2.40	-1.97	-2.36
10809399	---	---	-1.80	-2.95	-2.36
10779317	Ube2e2	ubiquitin-conjugating enzyme E2E 2 (UBC4/5 homolog, yeast	-1.43	-1.54	-2.36
10918600	Cgnl1	cingulin-like 1	1.19	-1.02	-2.36
10841416	Procr	protein C receptor, endothelial	-2.01	-2.26	-2.38
10830291	Frk	fyn-related kinase	-1.47	-1.69	-2.38
10781166	Clu	clusterin	-1.23	-1.60	-2.38
10702592	LOC683872 //	similar to Cd99 antigen-like 2 // similar to Cd99 antigen-like	-1.33	-1.56	-2.39
10891502	Galc	galactosylceramidase	-1.40	-1.79	-2.40
10864371	Frmd4b	FERM domain containing 4B	-1.65	-1.63	-2.40
10803440	---	---	-1.90	-1.73	-2.40
10770109	RGD1562462	similar to Ifi204 protein	-2.01	-2.06	-2.41
10763883	Klhdc8a	kelch domain containing 8A	-1.54	-2.01	-2.41
10775866	Areg	amphiregulin	-1.47	-1.33	-2.41
10736679	Tmem98	transmembrane protein 98	-2.33	-2.10	-2.42
10770795	Hsd11b1	hydroxysteroid 11-beta dehydrogenase 1	-1.37	-1.42	-2.43
10849943	Slc4a11	solute carrier family 4, sodium bicarbonate transporter-like, r	-2.07	-1.98	-2.43
10866215	Ly49s7	Ly49 stimulatory receptor 7	-2.13	-2.11	-2.43
10865782	Ccnd2	cyclin D2	-3.00	-2.91	-2.43
10860801	RGD1563091	similar to OEF2	-2.74	-2.71	-2.44
10799590	Frmd4a	FERM domain containing 4A	-2.32	-2.01	-2.44
10724042	P2ry2	purinergic receptor P2Y, G-protein coupled 2	1.02	-1.07	-2.44
10905988	Celsr1	cadherin, EGF LAG seven-pass G-type receptor 1 (flamingo h	-1.08	-1.01	-2.45
10797741	Aof1	amine oxidase, flavin containing 1	-1.72	-1.95	-2.45
10715416	Loxl4	lysyl oxidase-like 4	-3.38	-3.30	-2.45
10739977	RGD1308168	similar to chromosome 17 open reading frame 27	-1.19	-1.20	-2.45
10717141	Arhgap18	Rho GTPase activating protein 18	-1.38	-1.21	-2.46
10712853	Clcf1	cardiotrophin-like cytokine factor 1	-1.79	-1.91	-2.47
10851550	Wfdc5	WAP four-disulfide core domain 5	-2.15	-1.90	-2.48
10707707	Mtmr10	myotubularin related protein 10	-1.96	-2.02	-2.48
10780205	Mmp14	matrix metallopeptidase 14 (membrane-inserted)	-1.24	-1.16	-2.48
10938849	RGD1561931	similar to KIAA2022 protein	-2.00	-2.45	-2.49
10860947	Dlx5	distal-less homeobox 5	1.11	1.06	-2.49
10862561	Hoxa5	homeo box A5	1.40	1.61	-2.49
10731047	Ablim1	actin-binding LIM protein 1	-1.03	-1.19	-2.50
10917707	RGD1312026	similar to RIKEN cDNA C230081A13	-2.39	-2.73	-2.50
10829082	Abcg1	ATP-binding cassette, sub-family G (WHITE), member 1	-1.44	-1.33	-2.51
10860878	Pon3	paraoxonase 3	1.17	-1.09	-2.52
10707848	Lass3	LAG1 homolog, ceramide synthase 3	-1.98	-2.02	-2.52
10895406	Phlda1	pleckstrin homology-like domain, family A, member 1	-1.69	-1.52	-2.53
10877997	Cdkn2a	cyclin-dependent kinase inhibitor 2A	-10.26	-19.15	-2.54
10786401	Wnt5a	wingless-related MMTV integration site 5A	-1.77	-1.35	-2.54
10904528	Lynx1	Ly6/neurotoxin 1	1.06	1.07	-2.54
10862541	Hoxa1	homeo box A1	-1.09	-1.06	-2.55
10751896	Cldn1	claudin 1	1.39	1.49	-2.55
10904189	St3gal1	ST3 beta-galactoside alpha-2,3-sialyltransferase 1	-1.83	-1.71	-2.56
10724967	Dkk3	dickkopf homolog 3 (Xenopus laevis)	-2.66	-2.73	-2.57
10718666	Apeg3	antisense paternally expressed gene 3	-1.69	-1.64	-2.57
10901555	LOC679937	similar to CG4025-PA	-1.26	-1.39	-2.58
10846781	Tfpi	tissue factor pathway inhibitor	-3.13	-4.10	-2.58
10869253	Ugcg	UDP-glucose ceramide glucosyltransferase	-1.41	-1.51	-2.59

10818809	Bcar3	breast cancer anti-estrogen resistance 3	-1.26	-1.24	-2.60
10878001	Cdkn2b	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	-3.35	-4.23	-2.60
10735091	Cldn7	claudin 7	-1.56	-2.25	-2.60
10740209	Slc16a3	solute carrier family 16 (monocarboxylic acid transporters), n	-3.54	-2.36	-2.63
10727232	Tmem16a	transmembrane protein 16A	-1.42	-1.43	-2.63
10855934	RGD1565731	similar to KIAA1680 protein	1.01	1.07	-2.64
10829418	Pcbp3	poly(rC) binding protein 3	-2.33	-2.33	-2.64
10934098	Gpr165	G protein-coupled receptor 165	-1.64	-1.96	-2.65
10872336	Marcksl1	MARCKS-like 1	-2.78	-3.27	-2.65
10859781	Bicd1	bicaudal D homolog 1 (Drosophila)	-1.68	-2.92	-2.66
10714276	Trpm6	transient receptor potential cation channel, subfamily M, men	-1.87	-2.01	-2.67
10729074	Tle4	transducin-like enhancer of split 4, homolog of Drosophila E(s	-2.14	-1.80	-2.68
10876466	Gne	glucosamine	-1.47	-1.83	-2.68
10821408	Fst	follistatin	-4.35	-5.58	-2.69
10904533	Ly6d	lymphocyte antigen 6 complex, locus D	1.21	1.01	-2.70
10760738	Fam20c	family with sequence similarity 20, member C	-1.42	-1.38	-2.70
10859277	Gprc5a	G protein-coupled receptor, family C, group 5, member A	-1.23	-1.31	-2.70
10769672	Rgs4	regulator of G-protein signaling 4	-7.09	-8.12	-2.71
10723866	Dgat2	diacylglycerol O-acyltransferase 2	-1.84	-2.16	-2.71
10935353	Rab33a	RAB33A, member of RAS oncogene family	-1.39	-1.93	-2.72
10862547	Hoxa2	homeo box A2	1.08	-1.21	-2.73
10823254	RGD1562629	similar to neurobeachin	-1.26	-2.25	-2.73
10925291	Cxcr7	chemokine (C-X-C motif) receptor 7	-1.99	-1.86	-2.74
10804316	RGD1561988	similar to Colorectal mutant cancer protein (MCC protein)	-1.39	-1.83	-2.74
10865321	Fam80b	family with sequence similarity 80, member B	1.12	1.26	-2.75
10750513	Bace2	beta-site APP-cleaving enzyme 2	-1.28	-1.56	-2.75
10891684	Ccdc88c	coiled-coil domain containing 88C	-1.09	-1.06	-2.76
10827989	---	---	-2.04	-3.56	-2.78
10902080	Tmtc2	transmembrane and tetratricopeptide repeat containing 2	-2.38	-2.81	-2.78
10871339	Zswim5	zinc finger, SWIM domain containing 5	-1.45	-1.52	-2.78
10823252	RGD1562629	similar to neurobeachin	-1.33	-2.36	-2.79
10912255	Plod2	procollagen lysine, 2-oxoglutarate 5-dioxygenase 2	-2.96	-6.47	-2.79
10746387	Epn3	epsin 3	-1.14	-1.23	-2.80
10863430	Hk2	hexokinase 2	-1.15	-1.12	-2.81
10882317	G1p2	interferon, alpha-inducible protein (clone IFI-15K)	-2.13	-2.20	-2.82
10933716	Sat1	spermidine/spermine N1-acetyl transferase 1	1.30	-1.02	-2.83
10813992	LOC683212	similar to keratin complex 1, acidic, gene 18	-1.70	-2.76	-2.85
10917703	RGD1312026	similar to RIKEN cDNA C230081A13	-2.60	-2.92	-2.85
10838725	Chst14	carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 1	-1.07	-1.31	-2.86
10874728	Mxra8	matrix-remodelling associated 8	-2.33	-5.38	-2.86
10934118	Ar	androgen receptor	2.27	2.37	-2.89
10777137	Slit2	slit homolog 2 (Drosophila)	-2.22	-2.53	-2.90
10816026	Pdgfc	platelet-derived growth factor, C polypeptide	-2.21	-2.60	-2.91
10788462	Dlc1	deleted in liver cancer 1	-2.01	-2.11	-2.91
10706146	Rhpn2	rhophilin, Rho GTPase binding protein 2	-1.65	-2.61	-2.92
10851952	B4galt5	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypept	-1.20	-1.30	-2.93
10887306	Tnfaip2	tumor necrosis factor, alpha-induced protein 2	1.03	-1.15	-2.96
10775896	Cxcl2	chemokine (C-X-C motif) ligand 2	-1.42	-1.83	-2.96
10762442	Fbxo21	F-box protein 21	-1.50	-1.35	-2.97
10902280	Nav3	neuron navigator 3	-1.43	-1.60	-2.98
10867020	Fam80b	family with sequence similarity 80, member B	1.14	1.27	-3.00
10737429	Mmd	monocyte to macrophage differentiation-associated	-2.64	-3.21	-3.00
10761287	Gats	opposite strand transcription unit to Stag3	-1.82	-1.89	-3.02
10708954	Arrb1	arrestin, beta 1	-1.98	-1.93	-3.02
10793675	Ctla2a	cytotoxic T lymphocyte-associated protein 2 alpha	-2.86	-2.97	-3.04
10816405	Crabp2	cellular retinoic acid binding protein 2	-1.01	-1.51	-3.07
10880074	Tinagl1	tubulointerstitial nephritis antigen-like 1	-1.15	-1.54	-3.07
10804562	Nid67	putative small membrane protein NID67	-3.32	-3.85	-3.08
10928700	Abca12	ATP-binding cassette, sub-family A (ABC1), member 12	1.25	1.53	-3.08
10804415	Sema6a	sema domain, transmembrane domain (TM), and cytoplasmic	-2.58	-2.63	-3.09
10741330	Tmem204	transmembrane protein 204	-2.70	-2.88	-3.11
10719616	PVR	poliovirus receptor	-2.54	-2.80	-3.14
10911720	Hmgcll1	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase-like 1	-1.73	-3.29	-3.14
10802972	Mbp	myelin basic protein	-1.63	-1.77	-3.19
10935600	Vgll1	vestigial like 1 homolog (Drosophila)	-2.57	-2.96	-3.20
10790442	Ppyr1	pancreatic polypeptide receptor 1	-1.65	1.03	-3.21
10868871	Galnt12	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylga	-1.56	-2.17	-3.25
10932089	Slc6a14	solute carrier family 6 (neurotransmitter transporter), membe	-3.17	-1.93	-3.25
10902282	Nav3	neuron navigator 3	-1.38	-1.46	-3.26
10750848	Nfkbiz	nuclear factor of kappa light polypeptide gene enhancer in B-	-1.58	-1.55	-3.26
10884080	Lamb1	laminin, beta 1	-1.07	-1.26	-3.28
10869535	Tlr4	toll-like receptor 4	1.42	1.11	-3.28

10775573	Bmp3	bone morphogenetic protein 3	1.29	1.98	-3.31
10865222	Bid	BH3 interacting domain death agonist	-1.28	-1.45	-3.32
10732652	Dusp1	dual specificity phosphatase 1	-1.23	-1.38	-3.32
10939919	Fgf13	fibroblast growth factor 13	-3.20	-5.55	-3.32
10801262	Rell2	RELT-like 2	-2.19	-2.11	-3.35
10896948	Khdrbs3	KH domain containing, RNA binding, signal transduction asso	-2.44	-3.02	-3.35
10750672	St3gal6	ST3 beta-galactoside alpha-2,3-sialyltransferase 6	-1.43	-1.40	-3.35
10866222	Ly49s7	Ly49 stimulatory receptor 7	-3.08	-3.00	-3.36
10707303	RGD1564265	similar to integral membrane protein GDD1	-1.52	-2.34	-3.36
10906592	Slc38a1	solute carrier family 38, member 1	-1.24	-1.12	-3.38
10714528	Dmrt2	doublesex and mab-3 related transcription factor 2	-2.48	-4.05	-3.43
10890354	Cdkl1	cyclin-dependent kinase-like 1 (CDC2-related kinase)	-1.46	-1.64	-3.44
10907795	Gucy1a2	guanylate cyclase 1, soluble, alpha 2	-1.42	-1.35	-3.44
10775873	Ereg	epiregulin	-1.39	-2.06	-3.45
10777232	Cd38	CD38 molecule	-1.95	-2.77	-3.46
10836253	Galnt13	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylga	-3.41	-3.86	-3.48
10906679	Rapgef3	Rap guanine nucleotide exchange factor (GEF) 3	-2.47	-2.06	-3.50
10804463	Lox	lysyl oxidase	-2.64	-3.68	-3.52
10772002	Tmprss11e	transmembrane protease, serine 11e	-1.38	-1.53	-3.56
10897752	Mgat3	mannoside acetyl glucosaminyltransferase 3	-1.53	-2.23	-3.57
10924335	Plcd4	phospholipase C, delta 4	-2.82	-2.87	-3.61
10853871	Cftr	cystic fibrosis transmembrane conductance regulator homolo	-2.72	-3.43	-3.65
10820586	F2r	coagulation factor II (thrombin) receptor	-1.16	-1.36	-3.66
10916659	Oaf	OAF homolog (Drosophila)	-3.19	-3.73	-3.67
10919354	Pls1	plastin 1 (I isoform)	1.42	1.50	-3.67
10778505	Grb10	growth factor receptor bound protein 10	-4.00	-3.99	-3.68
10860815	Samd9l	sterile alpha motif domain containing 9-like	-6.34	-5.23	-3.71
10754735	Muc4	mucin 4	1.16	-1.30	-3.79
10781849	Lmo7	LIM domain only 7	-1.84	-2.22	-3.79
10860806	Samd9l	sterile alpha motif domain containing 9-like	-6.28	-5.18	-3.82
10860809	Samd9l	sterile alpha motif domain containing 9-like	-6.28	-5.18	-3.82
10860812	RGD1561472	similar to mKIAA2005 protein	-6.28	-5.18	-3.82
10750685	Col8a1	collagen, type VIII, alpha 1	1.00	-1.47	-3.84
10842143	Wfdc2	WAP four-disulfide core domain 2	-1.75	-3.22	-3.88
10883785	Odc1	ornithine decarboxylase 1	-3.47	-4.70	-3.91
10835449	Lamc3	laminin gamma 3	-2.09	-1.95	-3.93
10819269	Slc39a8	solute carrier family 39 (metal ion transporter), member 8	-1.58	-2.17	-3.95
10848370	Meis2	Meis1, myeloid ecotropic viral integration site 1 homolog 2	-1.49	-1.86	-3.96
10832563	Gstt3	glutathione S-transferase, theta 3	-1.08	-1.66	-4.01
10818892	Tram1l1	translocation associated membrane protein 1-like 1	-4.11	-5.62	-4.02
10788569	RGD1561067	similar to RNA binding protein gene with multiple splicing	-3.45	-4.55	-4.03
10704956	Plaur	plasminogen activator, urokinase receptor	-3.72	-4.53	-4.09
10929288	Serpine2	serine (or cysteine) peptidase inhibitor, clade E, member 2	-2.26	-1.96	-4.10
10813236	Sepp1	selenoprotein P, plasma, 1	1.29	-1.43	-4.14
10771389	Hpse	heparanase	1.09	-1.04	-4.15
10764551	Ptgs2	prostaglandin-endoperoxide synthase 2	-1.79	-1.71	-4.19
10752839	Adamts1	a disintegrin-like and metallopeptidase (reprolysin type) with	-2.57	-3.66	-4.21
10716454	Emx2	empty spiracles homeobox 2	-1.57	-1.72	-4.27
10867060	Tuba8	tubulin, alpha 8	-2.96	-4.22	-4.27
10827068	Bmpr1b	bone morphogenetic protein receptor, type 1B (mapped)	-1.57	-2.07	-4.28
10836446	Gca	grancalcin	-3.13	-5.13	-4.28
10924824	Sp100	nuclear antigen Sp100	-1.30	-1.22	-4.32
10802023	Arsi	arylsulfatase i	-3.14	-2.08	-4.38
10897529	Lgals1	lectin, galactose binding, soluble 1	-4.94	-6.59	-4.38
10772657	RGD1359713	hypothetical RNA binding protein RGD1359713	-1.38	-1.37	-4.42
10905974	Wnt7b	wingless-related MMTV integration site 7B	-1.69	-2.13	-4.42
10849833	Il1a	interleukin 1 alpha	-1.56	-1.52	-4.43
10924805	Sp140	SP140 nuclear body protein	-1.01	-1.36	-4.44
10871724	LOC679119	similar to bone morphogenetic protein 8b	-1.48	-1.75	-4.44
10742802	Sparc	secreted acidic cysteine rich glycoprotein	-3.63	-3.07	-4.46
10738051	Csf3	colony stimulating factor 3 (granulocyte)	-2.61	-3.18	-4.55
10763351	Serpinb11	serine (or cysteine) peptidase inhibitor, clade B (ovalbumin),	1.09	-1.44	-4.55
10808431	Irf8	interferon regulatory factor 8	-1.40	-1.50	-4.56
10891765	Tc2n	tandem C2 domains, nuclear	-2.95	-3.63	-4.57
10820282	Vcan	versican	-2.73	-3.49	-4.65
10939744	Gpc4	glypican 4	-1.59	-1.96	-4.67
10908037	Sesn3	sestrin 3	-2.20	-2.71	-4.71
10751239	Upk1b	uroplakin 1B	-5.18	-12.84	-4.73
10719000	---	---	-4.17	-4.19	-4.79
10821367	RGD1307506	similar to RIKEN cDNA 2310016C16	-3.33	-4.27	-4.80
10714900	Ifit2	interferon-induced protein with tetratricopeptide repeats 2	-1.08	-1.62	-4.82
10772013	Tmprss11b	transmembrane protease, serine 11b	-1.43	-1.37	-4.83

10865349	Slc2a3	solute carrier family 2 (facilitated glucose transporter), memt	-6.51	-5.30	-4.83
10849700	Mal	myelin and lymphocyte protein, T-cell differentiation protein	-1.09	-2.15	-4.87
10898268	Arhgap8	Rho GTPase activating protein 8	-1.63	-1.83	-4.90
10736784	Slfn5	schlafen 5	-3.68	-4.78	-4.94
10791250	Lpl	lipoprotein lipase	-2.87	-4.15	-5.02
10844223	Ptges	prostaglandin E synthase	-2.09	-2.28	-5.03
10936482	Timp1	tissue inhibitor of metalloproteinase 1	-1.32	-1.69	-5.05
10767388	Cd55	CD55 antigen	-2.14	-2.39	-5.14
10826846	Sgms2	sphingomyelin synthase 2	-1.88	-2.13	-5.23
10935087	Nrk	Nik related kinase	-4.33	-8.54	-5.28
10902047	Nts	neurotensin	-5.45	-5.82	-5.29
10798135	Serpinb9	serine (or cysteine) peptidase inhibitor, clade B, member 9	-3.36	-3.17	-5.31
10915843	Glb1l2	galactosidase, beta 1-like 2	-2.32	-2.48	-5.44
10797032	Dapk1	death associated protein kinase 1	-2.54	-3.83	-5.52
10784355	Ebpl	emopamil binding protein-like	-1.33	-1.66	-5.55
10824732	Sprr1al	small proline-rich protein 1A-like	-1.05	-1.83	-5.55
10906926	Rnd1	Rho family GTPase 1	-2.87	-2.94	-5.56
10866271	Styk1	serine/threonine/tyrosine kinase 1	-2.84	-2.51	-5.60
10747244	Krt13	keratin 13	1.55	1.50	-5.61
10917883	Loxl1	lysyl oxidase-like 1	-3.84	-6.91	-5.81
10749839	Vgll3	vestigial like 3 (Drosophila)	-3.52	-4.93	-5.85
10733509	Shroom1	shroom family member 1	-2.76	-3.29	-5.86
10780813	Fgf9	fibroblast growth factor 9	-2.15	-2.75	-5.89
10858370	Usp18	ubiquitin specific peptidase 18	-2.00	-3.55	-6.02
10775997	Slc4a4	solute carrier family 4 (anion exchanger), member 4	-2.27	-4.81	-6.04
10771998	RGD1559459	similar to Expressed sequence AI788959	1.14	-1.24	-6.13
10876069	Aqp3	aquaporin 3	-1.73	-1.65	-6.20
10898229	Parvb	parvin, beta	-2.53	-3.29	-6.25
10729635	Gldc	glycine decarboxylase	-4.48	-5.38	-6.26
10939866	Arhgef6	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6	-1.76	-2.42	-6.30
10937903	Gpr64	G protein-coupled receptor 64	-1.76	-2.16	-6.31
10890575	Six1	sine oculis-related homeobox 1 homolog (Drosophila)	-1.09	-1.51	-6.39
10830230	Smpdl3a	sphingomyelin phosphodiesterase, acid-like 3A	-1.74	-2.30	-6.44
10884478	Foxg1	forkhead box G1	-1.04	-1.30	-6.47
10884688	Pax9	paired box gene 9	-1.74	-1.97	-6.53
10821486	Isl1	ISL1 transcription factor, LIM/homeodomain 1	-1.41	-1.72	-6.60
10836588	Dhrs9	dehydrogenase/reductase (SDR family) member 9	-2.27	-2.12	-6.62
10741486	Msln	mesothelin	-1.65	-2.00	-6.68
10749484	Timp2	tissue inhibitor of metalloproteinase 2	-3.08	-3.35	-6.79
10939929	---	---	-6.63	-7.40	-6.95
10853396	Steap1	six transmembrane epithelial antigen of the prostate 1	-1.35	-1.68	-7.22
10938983	Cysltr1	cysteinyl leukotriene receptor 1	-3.82	-4.18	-7.29
10860481	Sema3a	sema domain, immunoglobulin domain (Ig), short basic doma	-2.91	-3.09	-7.31
10729777	Ch25h	cholesterol 25-hydroxylase	-2.23	-3.07	-7.31
10924245	Il8rb	interleukin 8 receptor, beta	-2.59	-2.89	-7.51
10903825	Has2	hyaluronan synthase 2	-14.69	-11.16	-7.94
10935843	---	---	-1.46	-3.46	-8.07
10795898	Chrm3	cholinergic receptor, muscarinic 3, cardiac	-4.95	-8.78	-8.54
10812589	F2rl2	coagulation factor II (thrombin) receptor-like 2	-5.86	-5.23	-8.60
10734382	Pmp22	peripheral myelin protein 22	-2.26	-4.08	-9.06
10761096	LOC367994	similar to uroplakin 3B isoform b	-2.03	-5.32	-9.12
10899405	Krt7	keratin 7	-3.02	-3.17	-10.15
10824726	Sprr1b	small proline-rich protein 1B	-1.47	-2.66	-10.59
10773115	Prom1	prominin 1	-2.41	-7.05	-10.60
10703838	---	---	-2.65	-3.27	-11.21
10932973	---	---	-3.72	-3.34	-11.30
10704127	Cdc42ep5	CDC42 effector protein (Rho GTPase binding) 5	-10.93	-13.19	-13.05
10839307	MGC105649	hypothetical LOC302884	-1.53	-3.57	-16.21
10815679	Mme	membrane metallo endopeptidase	-7.64	-16.69	-16.32
10867142	Eya1	eyes absent 1 homolog (Drosophila)	-6.22	-10.10	-17.06
10768332	Rgs2	regulator of G-protein signaling 2	-3.22	-4.16	-18.16
10751352	Krt8	keratin 8	-3.33	-4.26	-22.13
10702660	Akap12	A kinase (PRKA) anchor protein (gravin) 12	-7.43	-11.76	-22.67
10835817	Ptgs1	prostaglandin-endoperoxide synthase 1	-1.76	-2.07	-23.71
10866526	Arhgdib	Rho, GDP dissociation inhibitor (GDI) beta	-3.70	-2.46	-24.51
10906626	Slc38a4	solute carrier family 38, member 4	-8.50	-10.33	-28.77
10763359	Serpinb7	serine (or cysteine) peptidase inhibitor, clade B, member 7	-4.09	-5.06	-43.87
10907524	Krt8	keratin 8	-3.93	-4.68	-54.09

* Fold Change > 2.0, Adjusted p-value < 0.05

Supplementary Table 3 - List of genes up- and down- regulated in TECs after the 1st and 2nd skin transplantation. List of genes differentially expressed in 3 types of comparisons: sr1-TECs *versus* TECs, sr2-TECs *versus* TECs and HF cells *versus* TECs. * Fold Change > 2.0, Adjusted p-value < 0.05.

Supplementary Table 4. Selected list of genes up- and down- regulated after the 1st and the 2nd transplantation

Probe set ID	Gene name		sr1-TECs *vs* TECs *	sr2-TECs *vs* TECs *	HF cells *vs* TECs *
10890575_at	Six1	sine oculis-related homeobox 1	-1.1	-1.5	-6.4
10867142_at	Eya1	eyes absent 1	-6.2	-10.1	-17.1
10840555_at	Pax1	paired box gene 1	-2.2	-2.5	-2.2
10884688_at	Pax9	paired box gene 9	-1.7	-2	-6.5
10735091_at	Cldn7	claudin 7	-1.6	-2.3	-2.6
10751896_at	Cldn1	claudin 1	1.4	1.5	-2.6
10899405_at	Krt7	keratin 7	-3	-3.2	-10.2
10747244_at	Krt13	keratin 13	1.6	1.5	-5.6
10813992_at	LOC683212	keratin complex 1, acidic, gene 18	-1.7	-2.8	-2.8
10907524_at	Krt8	keratin 8	-3.9	-4.7	-54.1
10751352_at	Krt8	keratin 8	-3.3	-4.3	-22.1
10907468_at	Krt1	keratin 1	1.5	2.5	1.8
10747084_at	Krt10	keratin 10	1.7	3.5	5.6
10747252_at	Krt15	keratin 15	3.3	2.1	1.6
10705654_at	Lgals7	lectin, galactose binding, soluble 7	35.4	39.7	72.2
10752295_at	Tbx1	T-box 1	2.8	2.8	13.3
10835958_at	Lhx2	LIM homeobox protein 2	2	2.1	3.3
10781378_at	Hr	hairless	2.5	3	5.9
10796220_at	Gata3	GATA binding protein 3	3.2	3.5	3.7

* Fold Change > 2.0, Adjusted p-value < 0.05

Supplementary Table 4 - Selected list of genes up- and down- regulated after the 1st and 2nd skin transplantation. A complete list (641 genes) is given in Supplementary Table 3.

Supplementary Table 5. List of primers used for RT-PCR

Gene name	Forward primer 5'–3'	Reverse primer 5'–3'
Actb	TCATGTTTGAGACCTTCAACACCC	GTACTTGCGCTCAGGAGGAG
AIRE	AGCAAGTATTCGAGTCAG	CGCATTCATCCTCGTT
Blimp1	GACGGGGGTACTTCTGTTCA	TGGGGACACTCTTTGGGTAG
Bmi1	TGTGCGTTACTTGGAGACCA	TCATTCACCTCCTCCTTTGG
CDP	TCGGCAGGTCAAAGAGAAGT	CACAGATTCGCTGGAACTCA
Foxn1	CACTGGAAGCCTTTGAGGAG	AGATGGGCTTTGGGAAGAGT
Gal-7	ACCCATCACAAGACCCCTCT	ATCCCCGATCACAGTCT
Gata3	TACCACCTATCCGCCCTATG	GTCTGACAGTTCGCACAGGA
Ihh	GTCAGCTGTGAAGCCAGGA	CCTCGTGAGAGGAGCATAGG
Ivl	CAGCAACCACAAGAACAGGA	AGTTCTGGCTCTGGGGACTT
Krt13	TTCGTGACTGGCATCTGAAG	GTATCCTCCAATGCTGCCAT
Krt14	TGAGAGCCTCAAGGAAGAGC	CAGTAACGGCCTTTGGTCTC
Krt19	ACACCAGGCATTGACCTAGC	GTGTCAGCACGCACGTTACT
Krt4	AGCTCATGCAGGACAGTGTG	AAGATGCCTACACCAAACGC
Krt7	TGACGTCAAAGCCCAGTATG	GGTAGGTGGCGATCTCAATG
KrtHa2	AGCAGACACCAATGGCCTAC	GCCACCTGCTTGTTAAGCTC
Krox20	CCAAGGCCGTAGACAAAATC	GAAGAGGCTGTGGTTGAAGC
Lef1	ACATCCCGTCAGAGGTCAAC	TGAGGCTTCACGTGCATTAG
Lgr5	AATGGTGCCTCGCAAATTAC	GAAAGCGTTGGGGTGAATAA
Trp63	ACTGCCAGATTGCAAAGACC	GCTGCTGTTGCACATGAAAT
Shh	GGAAGATCACAAGAAACTCCGAAC	GGATGTGAGCTTTGGATTCATAGTAG
Six1	CGTGGGCAAATATCGG	GTTATTGTTTTCGGTGTTCT
Spink5	CCCCAGCAAAGTTGAATTGT	CTCTGGCTTTTCGCATTCTC
Plzf	AAAGCAGAGGACCTGGATGA	TCATGGCTGAGAGACCAAAA
Pax1	GGGCCATACGAAGCAAGTAA	CAAGGCAGGTTTCTCGAGTC
Pax9	AAGAAGCCAAGTACGG	TGCATTCCCTTGAAAGC
Hoxa3	TGTCTCTCCCCCTCAAAGTG	AGGTAGCGGTTGAAGTGGAA
PLET1	GTGACAGTCCCTGTGAACGA	ACACTGTAGCTGTGCGGTTG

Supplementary Table 6. List of primers used for q-PCR

Gene name	Forward primer 5'–3'	Reverse primer 5'–3'
Eya1	GGGTCCTACGCCAACAGATA	GGTCCTGTCCATTGTCGTCT
Foxn1	CCCATCTACTCCTACAGC	TCTCTACCTTCTCAAAGCAC
KerHa2	GAGTGTTGACTGTTCGT	CCGCCTTAATATCGAGGT
Gal-7	ACCCATCACAAGACCCCTCT	ATCCCCGATCACAGTCT
Gata3	GAGAGCAGGGACTTCCTGTG	CATCATGCACCTTTTTGCAC
HPRT	GGTCCATTCCTATGACTG	CAACCTTAACCATTTTGG
Pax1	GGGCCATACGAAGCAAGTAA	CAAGGCAGGTTTCTCGAGTC
Pax9	AAGAAGCCAAGTACGG	TGCATTCCCTTGAAAGC