Genome-edited skin epidermal stem cells protect mice from cocaine-seeking behaviour and cocaine overdose

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Cocaine addiction is associated with compulsive drug seeking, and exposure to the drug or to drug-associated cues leads to relapse, even after long periods of abstention. A variety of pharmacological targets and behavioural interventions have been explored to counteract cocaine addiction, but to date no market-approved medications for treating cocaine addiction or relapse exist, and effective interventions for acute emergencies resulting from cocaine overdose are lacking. We recently demonstrated that skin epidermal stem cells can be readily edited using CRISPR (clustered regularly interspaced short palindromic repeats) and then transplanted back into the donor mice. Here, we show that the transplantation, into mice, of skin cells modified to express an enhanced form of butyrylcholinesterase—an enzyme that hydrolyses cocaine—enables the long-term release of the enzyme and efficiently protects the mice from cocaine-seeking behaviour and cocaine overdose. Cutaneous gene therapy through skin transplants that elicit drug elimination may offer a therapeutic option to address drug abuse.

Drug addiction is a brain disorder characterized by compulsive drug seeking and taking, and a high likelihood of relapse when exposed to drugs or drug-associated cues1–3. Cocaine is a commonly abused drug, and current medications do not meet the urgent needs for treating ongoing cocaine use, relapse or acute emergencies that result from cocaine overdose4–6. Butyrylcholinesterase (BChE) is a natural enzyme that is present in hepatocytes and plasma, and it hydrolyses its normal substrate acetylcholine7–9. BChE can also hydrolyse cocaine at low catalytic efficiency into benzoic acid and ecgonine methyl ester, which are low in toxicity and rewarding properties10. Protein engineering has greatly enhanced the catalytic potency and substrate specificity of human BChE (hBChE) for cocaine hydrolysis10–13. The modified hBChE has more than 4,400 times higher catalytic efficiency than the wild-type (WT) enzyme with significantly reduced activity for acetylcholine14. However, the recombinant hBChE protein has to be delivered via a parenteral route and has a very short half-life in vivo7,8,15, making it potentially useful only for the acute treatment of cocaine overdose. Butyrylcholinesterase is readily available for extraction of epidermal stem cells through well-established protocols16–20. Skin transplants are also easily monitored for potential off-target effects of gene targeting, and removal of skin implants is relatively uncomplicated. (2) Epidermal stem cells can be readily engineered in vitro, and standard procedures exist for transplantation of the differentiated and stratified skin tissue back onto donor patients21–22. Compared with viral packaging, autologous skin grafts are inexpensive; skin transplantation is minimally invasive and has been used to treat burn patients for decades23–25. (3) Skin epidermal keratinocytes have low immunogenicity as Langerhans cells are the only cell subset that expresses major histocompatibility complex (MHC) class II for antigen-presentation antigens in healthy skin26. (4) Other examples of therapeutic large proteins secreted by skin epidermal cells have been reported (for example, apolipoprotein E and the blood clotting factor VIII and factor IX) that can cross the dermal barrier and enter the blood circulation for a systemic therapeutic effect27–31. Thus, cutaneous gene therapy can be used as a safe and effective way to treat non-skin diseases including drug abuse—a scenario that has not been explored before. In this report, we demonstrate key evidence that engineered skin transplants can efficiently deliver BChE in vivo and protect against cocaine seeking and overdose.

Results
As the methodology of this study is similar to that in our previous work in ref. 20, some text in this section has been adapted from that publication.

CRISPR-edited epidermal stem cells can express engineered hBChE for cocaine hydrolysis. To carry out clustered regularly interspaced short palindromic repeats (CRISPR)–mediated genome editing in mouse epidermal stem cells, we developed DNA vectors encoding the D10A mutant of CRISPR associated protein 9 (Cas910,11)32, two guide RNAs targeting the mouse Rosa26 locus and a Rosa26-targeting vector. The targeting vector contains two
homology arms for the Rosa26 locus, flanking an expression cassette that encodes the modified hBChE gene (Fig. 1a). Primary epidermal basal cells were isolated from newborn mice and electroporated with the Rosa26-targeting vector together with plasmids encoding Cas9 and Rosa26-specific guide RNAs. Clones were isolated on selection and the correct integration to the Rosa26 locus was confirmed by both PCR screening and Southern blotting analysis (Fig. 1b). Engineered epidermal cells exhibited robust expression and secretion of hBChE as shown by immunobLOTS and enzyme-linked immunosorbent assay (ELISA) (Fig. 1c,d). The secreted hBChE protein was functional as the conditioned medium collected from hBChE-expressing cells but not the control cells significantly induced degradation of cocaine in vitro (Fig. 1e). Consistent with previous reports, similar mutations in mouse BChE lead to only residual activity in cocaine hydrolysis (Fig. 1f and Supplementary Fig. 1a). To investigate the potential therapeutic effect of hBChE expression in vivo, we transplanted the organoids to isogenic host animals (CD1 and C57BL/6) (Fig. 2a,b). No significant

Engraftment of engineered epidermal stem cells can protect mice from cocaine-seeking behaviour and cocaine overdose. To efficiently transplant mouse epidermal stem cells, we developed an organotypic culture model with mouse epidermal stem cells in vitro by culturing the cells on top of acellularized mouse dermis. To efficiently transplant mouse epidermal stem cells, we developed an organotypic culture model with mouse epidermal stem cells in vitro by culturing the cells on top of acellularized mouse dermis. To efficiently engraft mouse epidermal stem cells, we developed an organotypic culture model with mouse epidermal stem cells in vitro by culturing the cells on top of acellularized mouse dermis. To efficiently engraft mouse epidermal stem cells, we developed an organotypic culture model with mouse epidermal stem cells in vitro by culturing the cells on top of acellularized mouse dermis. To efficiently engraft mouse epidermal stem cells, we developed an organotypic culture model with mouse epidermal stem cells in vitro by culturing the cells on top of acellularized mouse dermis.
rejection of the skin grafts was observed for at least five months after transplantation, suggesting that the targeted epidermal stem cells are well tolerated immunologically in vivo. Grafted skin exhibited normal epidermal stratification, proliferation and cell death (Fig. 2c and Supplementary Fig. 2b,c). The mice that were grafted with hBChE-expressing cells were stable for more than 10 wk (Fig. 2d). Consistent with previous observations31–36, our results confirm that a skin-derived therapeutic protein can cross the basement membrane barrier and enter the circulation in vivo.

Cocaine can block dopamine reuptake and elevate extracellular levels of dopamine, resulting in locomotor stimulation and reward-related behaviours1,3,41. The expression of BChE in mice grafted with hBChE-expressing cells (GhBChE mice) presumably removes cocaine much more quickly in vivo, leading to reduced extracellular dopamine and locomotor activity. We measured dopamine and cocaine levels in both GhBChE mice and control

Fig. 2 | Engraftment of hBChE-expressing cells can reduce cocaine-induced locomotion and protect against cocaine overdose. a, Skin organoids were developed from control or hBChE-producing cells and transplanted to the host mice. Cells were infected with lentivirus encoding firefly luciferase before engraftment to allow intravital imaging of the skin grafts. b, Histological examination of grafted skin collected from mice grafted with control (GWT) or hBChE skin organoids (GhBChE). Scale bar, 50 μm. c, Sections of grafted skin were immunostained with different antibodies (keratin 10 (Krt10), a marker for early epidermal differentiation; loricrin (Lor), a marker for late epidermal differentiation; and β4-integrin (CD104) (β4), a marker for skin basement membrane) as indicated. Dashed lines denote the basement of skin. DAPI, 4',6-diamidine-2'-phenylindole dihydrochloride; Der, dermis; Epi, epidermis. Scale bar, 50 μm. d, Mice were grafted with control (GWT) or hBChE skin organoids (GhBChE). The presence of hBChE in the blood was determined by ELISA for 10 wk after engraftment (n=5 mice in each group). e, Cocaine pharmacokinetics in the nucleus accumbens after i.p. administration of 10 mg kg−1 cocaine in GhBChE and GWT mice (n=6 for each group). Data are plotted as means ± s.e.m. A two-compartment in vivo pharmacokinetic model was built to represent the cocaine concentration–time profile in the nucleus accumbens. f, Changes in dopamine levels in the nucleus accumbens after i.p. administration of 10 mg kg−1 cocaine in GhBChE and GWT mice (n=6 for each group). Individual lines for each animal were plotted. Treatment × time interaction: F11,108 = 3.65, P < 0.0001, two-way ANOVA. g, h, Cocaine-induced locomotor activity in GhBChE and GWT mice (n=11 for GWT; n=8 for GhBChE). Non-linear dose versus response curves were simulated to represent cocaine-induced dose–response locomotor activity. g, Total distance travelled after 0, 10, 20 and 40 mg kg−1 cocaine i.p. injection. Treatment × doses interaction: F7,77 = 11.83, P < 0.0001, two-way ANOVA. h, Stereotypical counts after 0, 10, 20 and 40 mg kg−1 cocaine i.p. injection. Treatment × doses interaction: F7,77 = 6.223, P < 0.0009, two-way ANOVA. i, Lethality rates after injection of 40, 80, 120 and 160 mg kg−1 cocaine in GhBChE and GWT mice (n=3 independent experiments; for each experiment, 8 animals were examined in each test group). Individual data points represent the result from each experiment. Error bars represent s.e.m. and the central measure represents the mean.
mice grafted with WT epidermal cells (GWT mice) after an acute cocaine injection. We performed microdialysis in the nucleus accumbens of freely moving mice (Supplementary Fig. 2d), and the dialysates collected were quantified by liquid chromatography–mass spectrometry (LC-MS)41. As expected, GhBChE mice exhibited a much faster cocaine clearance (Fig. 2e) and less extracellular dopamine (Fig. 2f) in the nucleus accumbens than WT mice. Consistently, LC-MS analysis demonstrated a significantly faster clearance of benzoylecgonine (a major cocaine metabolite) in the plasma of GhBChE mice (Supplementary Fig. 2e). Engineered hBChE has very low activity for acetylcholine41. We did not detect significant differences in plasma acetylcholine levels in GhBChE and control mice (Supplementary Fig. 2f). To further assess the pharmacodynamics properties of cocaine in GhBChE mice, we monitored acute cocaine-induced locomotor behaviour. Both GhBChE and GWT mice exhibited dose–response relationships, as measured by the distance travelled and stereotypical counts, after cocaine administration (Fig. 2g,h). However, GhBChE mice showed significantly less distance travelled and lower stereotypical counts than GWT mice, as revealed by two-way analysis of variance (ANOVA). Together, our data strongly suggest that skin-derived hBChE can effectively hydrolyse cocaine and reduce extracellular levels of dopamine in grafted mice without significant effects on acetylcholine.

To determine whether engrafting hBChE-expressing cells protects mice from the acute systemic toxicity of cocaine, we delivered different doses of cocaine to grafted mice and calculated the lethality rates of cocaine. Doses of 40, 80, 120 and 160 mg kg\(^{-1}\) of cocaine had nearly 0 lethality in GhBChE mice, whereas 80 mg kg\(^{-1}\) of cocaine induced roughly 50% lethality and 120 and 160 mg kg\(^{-1}\) cocaine induced 100% lethality in GWT mice (Fig. 2i and Supplementary Video). A parallel test was conducted to test the toxicity of a related stimulant, methamphetamine, in GhBChE and GWT mice. There was no difference in the lethality induced by various doses of methamphetamine between GhBChE and GWT mice (Supplementary Fig. 2g). This finding suggests that the engraftment of hBChE-expressing cells can protect mice from the toxicity of cocaine overdose.

We then assessed protection against the development of cocaine-seeking behaviour using the conditioned place preference (CPP)43,44 paradigm, which is thought to model reward learning and seeking because experimental animals approach and remain in contact with cues that have been paired with the effects of the reward. We grafted hBChE-expressing cells to cocaine-naïve mice and used GWT animals as controls. After 4 d of place conditioning, GWT mice spent significantly more time in environments previously associated with cocaine, whereas GhBChE mice showed no such preference (Fig. 3a). As an additional control, the ethanol CPP was measured after 4 d of conditioning in GhBChE and GWT mice. In contrast, both GhBChE and GWT mice spent significantly more time in ethanol-paired environments (Fig. 3b). This finding indicates that the engraftment of hBChE-expressing cells efficiently and specifically attenuates the cocaine-induced rewarding effect.

To determine whether engrafting hBChE-expressing cells affects cocaine-induced reinstatement of drug seeking, we engraved hBChE-expressing cells in mice that previously acquired cocaine CPP. Following 10 d of recovery, we performed extinction training and drug-elicted reinstatement43,44. After a priming dose of cocaine injection, the preference for the previously cocaine-associated environment was restored in the GWT mice but not in the GhBChE mice (Fig. 3c). Because hBChE expression did not prevent CPP induction by ethanol (Fig. 3b), we used these GhBChE and GWT mice and performed extinction training followed by reinstatement. In contrast with those induced by cocaine, the ethanol CPP was similarly reinstated in both GhBChE and GWT mice (Fig. 3d). These results suggest that skin-derived hBChE efficiently and specifically disrupts cocaine-elicited reinstatement.

Engineered human epidermal stem cells can deliver hBChE in vivo. To test the feasibility of cutaneous gene therapy with human epidermal stem cells, we cultured human skin organoids from primary epidermal keratinocytes isolated from human newborn foreskin. To perform CIRPSR-mediated genome editing in human cells, we developed vectors encoding two guide RNAs targeting the human adeno-associated virus integration site 1 gene (AAVS1) locus and an AAVS1-targeting vector (Fig. 4a) that harbours the
expression cassette encoding engineered hBChE. Human epidermal keratinocytes were electroporated with the targeting vector together with plasmids encoding Cas9 and the guide RNAs. Clones were isolated and the correct integration confirmed by Southern blotting analysis (Fig. 4b). As with mouse cells, engineered human epidermal cells exhibited strong hBChE production, as determined by immunoblots and ELISA (Fig. 4c,d). Expression of the hBChE protein in human cells did not significantly change cell proliferation (Supplementary Fig. 3a) or differentiation (Supplementary Fig. 3b) in vitro. The engineered cells stratified and formed skin organoids in vitro, which were transplanted to nude hosts (Fig. 4e). Grafted skin exhibited normal epidermal stratification, proliferation and apoptosis in vivo (Fig. 4f and Supplementary Fig. 3c,d). Together, these results indicate that CRISPR editing of human epidermal stem cells does not significantly alter cellular dynamics and persistence in vivo. ELISA confirmed that the mice with engraftment of hBChE-expressing cells had significant levels of hBChE in the blood, and this expression was stable for more than 8 wk in vivo (Fig. 4g). Our results suggest the potential clinical relevance of cutaneous gene delivery for the treatment of cocaine abuse and overdose in the future.

Discussion
Our study demonstrates that transplantation of genome-edited skin stem cells can be used to deliver an active cocaine hydrolase long term in vivo. Skin epidermal stem cells can be successfully employed for ex vivo gene therapy, as efficient genetic manipulation is possible with minimal risk of tumorogenesis or other adverse events in vivo. Skin transplantation protocols, including procedures based on the use of human epidermal stem cells to generate cultured epidermal autografts, have been in clinical use for decades in the treatment of burn wounds. Engineered skin stem cells and cultured epidermal autografts have also been used to treat other skin diseases, including vitiligo and skin genetic disorders, such as epidermolysis bullosa. These regenerated skin grafts are stable and have been shown to survive long term in clinical follow-up studies. As such, the cutaneous gene therapy is long lasting, minimally invasive and safe. For cocaine addicts and individuals
with a potentially high risk of cocaine abuse who seek help or treatment, the cutaneous gene therapy approach with hBChE expression can address several key aspects of drug abuse, including reducing the development of cocaine-seeking behaviour, preventing cocaine-induced reinstatement of drug-seeking behaviour and protecting against cocaine overdose after skin transplantation, potentially making them 'immune' to further cocaine abuse. Because of the extremely high catalytic efficiency and high levels of hBChE, this approach can be highly efficient in protecting against a wide range of cocaine doses with little individual variation. It remains possible that the protective effect of hBChE-expressing skin grafts can be surmountable if extremely high doses of cocaine or other psychostimulants are used. For instance, it has been shown that the longer-acting mutant form of bacteria cocaine esterase can prevent cocaine-induced toxicity and ongoing intravenous self-administration in rodents, but the effects are diminished when high doses of cocaine are used. Additionally, the development of drug abuse is accompanied by learned association between drug effects and environmental cues, which plays a significant role in cocaine craving and relapse. Although hBChE is a highly potent cocaine hydrolase, it is unlikely to affect cue-induced relapse. It is also noteworthy that cocaine intake is not under the control of the test animals in our CPP model. Thus, future studies will be essential to determine the protective effect of hBChE, particularly in response to extremely high doses of cocaine, and to test the prevention of cue-induced relapse.

Cutaneous gene delivery with engineered epidermal stem cells may provide therapeutic opportunities for drug abuse or co-abuse beyond cocaine. For instance, glucagon-like peptide 1 (GLP1) is a major physiological incretin that controls food intake and glucose homeostasis. Several GLP1 receptor agonists have been approved by the Food and Drug Administration to treat type II diabetes. Our recent study indicates that skin-derived expression of GLP1 can effectively correct diet-induced obesity and diabetes in mice. Interestingly, GLP1 receptor agonists can also attenuate the reinforcing properties of cocaine, alcohol and nicotine in rodents. Thus, future studies will determine whether the expression of GLP1 from skin transplants can reduce cocaine, alcohol and nicotine use and relapse in patients with cocaine abuse, alcohol use disorder and nicotine dependence. Additionally, it will be important to investigate whether co-expression of hBChE and GLP1 in skin can be used to treat alcohol and/or nicotine co-abuse with cocaine, which occurs with high frequency and significantly increases the risk of drug-related morbidity and mortality.

The immune system recognizes and reacts against foreign antigens, including those arising from gene therapy-derived products. If an immune response to these products is triggered, neutralizing antibodies are produced that prevent the function of the therapeutic vector. If an immune response to these products is triggered, neutralizing antibodies are produced that prevent the function of the therapeutic vector. If an immune response to these products is triggered, neutralizing antibodies are produced that prevent the function of the therapeutic vector. If an immune response to these products is triggered, neutralizing antibodies are produced that prevent the function of the therapeutic vector.
Cell cycle analysis. Propidium iodide staining followed by flow cytometry assay were used to determine the effect of cell cycle profiles. Mouse and human epidermal cells were cultured in 2 cm cell culture dishes for 24 h, respectively. Cells were trypsinized and 1 x 10⁶ cells from each dish were collected, followed by a phosphate buffered saline (PBS) wash. Fixation of cells was carried out using 70% (v/v) ice-cold ethanol for 1 h. Then, the fixed cells were centrifuged at 500 g at 4 °C for 10 min, followed by two PBS washes. The cells were then treated with 75% (v/v) RNAse A in 100 μl PBS and incubated at 37 °C for 1 h. After incubation, the cells were collected by centrifugation at 500 g for 10 min, followed by another PBS wash. The cell pellet was re-suspended in 200 μl PBS, with the addition of propidium iodide solution at a final concentration of 25 ng/ml. After staining, the cells were analysed immediately using a BD FACSCanto II flow cytometer (BD Biosciences) with an excitation wavelength at 488 nm and emission at 585 nm. DNA content and histograms of cell cycle distribution were analysed using FlowJo software, version 10.

Protein biochemical analysis. Western blotting was performed as described previously70. Briefly, equal amounts of the cell lysates were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis and electroblotted onto a nitrocellulose membrane. The immunoblot was incubated with Odyssey blocking buffer (LI-COR Biosciences) at room temperature for 1 h, followed by overnight incubation with primary antibody. Blots were washed three times with Tween 20/Tris-buffered saline and incubated with a 1:10000 dilution of secondary antibody for 1 h at room temperature. Blots were washed three times with Tween 20/Tris-buffered saline again. Visualization and quantification were carried out with the LI-COR Odyssey scanner and software (LI-COR Biosciences).

Cocaine-induced behaviours. For all behavioural experiments except where noted, C57BL/6J mice were used. Roughly equal numbers of adult male and female mice were group housed until surgery. Mice were maintained under controlled temperature and humidity conditions on a 12 h:12 h light:dark cycle (lights on at 7:00). Water and food were available ad libitum. Mice weighed around 25–30 g at the beginning of the experiments. All procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Chicago Institutional Animal Care and Use Committee.

Drug. Cocaine hydrochloride and methamphetamine hydrochloride (Sigma–Aldrich) were dissolved in sterile saline and delivered intraperitoneally at a density that was not different from that of the pre-test on two consecutive days. On the following day of the last extinction, mice underwent reinstatement procedures, in which mice that were trained for cocaine CPP received an i.p. injection of 15 mg kg⁻¹ cocaine, and mice that were trained for ethanol CPP received an injection of 1 g kg⁻¹ ethanol. Immediately following injection, mice were allowed to explore the entire chambers for 20 min and the time spent in each area was recorded.

Acute drug overdose test. Two weeks after grafting surgery, 4 groups of GhBChE and 4 groups of GWT mice (n=8 in each group) received i.p. injections of cocaine at 40, 80, 120 and 160 mg kg⁻¹. As a control, 4 groups of GhBChE and 4 groups of GWT mice (n=8 in each group) received i.p. injections of methamphetamine at 34, 68 (LD50), 100 and 160 mg kg⁻¹. Two each of GhBChE and GWT mice with CD1 mice as hosts were also used to videotape and analyse stereotypical behaviours. Mice were monitored for 2 h following injection and the percent cocaine- and methamphetamine-induced lethality was calculated.

Specific methods. Figure 3a: one group of GhBChE and one group of GWT mice (n=8 in each group) were trained for cocaine CPP 7 d after engraftment. Mice underwent pre-testing on day 1, 4 d of cocaine conditioning from day 2 to day 5, and CPP testing on day 6. Figure 3b: one group of GhBChE and one group of GWT mice (n=8 in each group) were trained for ethanol CPP 7 d after engraftment. Mice underwent pre-testing on day 1, 4 d of ethanol conditioning from day 2 to day 5, and CPP testing on day 6. Figure 3c: two groups of drug-naïve WT mice (n=8 in each group) were trained for cocaine CPP from day 1 to day 6. On the following day, mice underwent engrafting surgery. The behavioural procedure resumed after engraftment surgery from day 18. Extinction was performed from day 18 to day 31. On day 32, mice underwent reinstatement induced by i.p. injection of cocaine. Figure 3d: one group of GhBChE and one group of GWT mice (n=8 in each group) were trained for ethanol CPP from day 1 to day 6. Reinstatement was performed from day 7 to day 20. On day 21, mice underwent reinstatement induced by i.p. injection of ethanol.

Statistical analysis. Statistical analysis was performed using Excel or OriginLab software. Box plots were used to describe the distribution without assumptions on the statistical distribution. A Student's t-test was used to assess the statistical significance (P value) of differences between two experimental conditions. For cocaine behavioural analysis, CPP results were analysed using repeated-measures ANOVA with within factor time (testing days) and between factor treatment (engraffment). Significant effects were further analysed with Fisher's t-tests.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.
Data availability

The authors declare that all data supporting the findings of this study are available within the paper and its Supplementary Information. Source data for Figs. 2 and 3 are available in FigurePaper at https://figshare.com/s/8983bad26b10a3db813.

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Author contributions
X.W. and M.X. designed the experiments. Y.L., Q.K., J.Y. and X.G. performed the experiments. Y.L., Q.K., J.Y., M.X. and X.W. analysed the data. X.W. and M.X. wrote the manuscript. All authors edited the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a | Confirmed

☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

☐ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐ The statistical test(s) used AND whether they are one- or two-sided

• Only common tests should be described solely by name; describe more complex techniques in the Methods section.

☐ A description of all covariates tested

☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

☐ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)

☐ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted

• Give P values as exact values whenever suitable.

☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

☐ Clearly defined error bars

• State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

| Data collection | Flowjo, Version 10. |
|-----------------|---------------------|

| Data analysis   | Excel 2010, originlab 2016, flowjo version 10. |
|-----------------|-----------------------------------------------|

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The authors declare that all data supporting the findings of this study are available within the paper and its supplementary information. Source data for Figures 2 and 3 are available in Figshare: https://figshare.com/s/898c3ab26b10a3d08b13 (ref. 63).
Field-specific reporting
Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences   ☐ Behavioural & social sciences   ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design
All studies must disclose on these points even when the disclosure is negative.

| Sample size | Based on mean and standard deviation data from our previous studies, a biostatistician, Kristin Wroblewski, from the Biostatistics Core at the Department of Health at our university, performed a sample-size determination for the project. |
| Data exclusions | No data were excluded. |
| Replication | Experiments were reliably reproduced. |
| Randomization | For test animals, we used the same C57Bl/6j or CD1 mice at a similar age. Roughly equal numbers of adult male and female mice were used in the analysis. Animals were randomly allocated to the experimental groups. |
| Blinding | Transplanted mice were blind to investigators performing the experiments until data analysis was completed. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
| ☑ | Unique biological materials |
| ☑ ☑ | Antibodies |
| ☑ | Eukaryotic cell lines |
| ☑ | Palaeontology |
| ☑ | Animals and other organisms |
| ☑ | Human research participants |

Methods

| n/a | Involved in the study |
| ☑ | ChIP-seq |
| ☑ | Flow cytometry |
| ☑ | MRI-based neuroimaging |

Antibodies

| Antibodies used | Antibody used: Guinea pig anti-K5 (gift from Dr. Elaine Fuchs), rabbit anti-K14, anti-K10 and loricrin antibody (gifts from Dr. Elaine Fuchs). Anti-beta4-integrin (Rat monoclonal, BD 553745, from BD Pharmigen). Ser Pho-histone antibody (EMD Millipore). Cleaved caspase 3: cell signaling technology, BCHE antibody: Proteintech. |
| Validation | All antibodies were validated by immunoblots or immuofluorescence stainings with proper negative controls to detect mouse and human proteins. |

Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) | Mouse primary keratinocytes were isolated from newborn mouse skin. Human newborn keratinocytes were purchased from Invitrogen/Life Science. |
| Authentication | Only primary cells were used. No authentication was used. |
| Mycoplasma contamination | All the cells were tested and determined not to be contaminated with mycoplasma. |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used in this study. |
Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

| Category                  | Description                                                                 |
|---------------------------|-----------------------------------------------------------------------------|
| Laboratory animals        | Roughly equal numbers of adult male and female (CD1 or C57/B6) mice were used. |
| Wild animals              | The study did not involve wild animals                                       |
| Field-collected samples   | The study did not involve samples collected from the field.                 |

Flow Cytometry

Plots

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Propidium Iodide (PI) staining followed by Flow Cytometry Assay were used to determine the effect of cell-cycle profiles. Mouse and human epidermal cells were cultured in two 6-cm cell-culture dishes for 24 hours, respectively. Cells were trypsinized and 1x10^5 cells from each dish were collected, followed by one PBS wash. Fixation of cells was carried out using 70% (v/v) ice cold ethanol for 1 hour. Then, the fixed cells were centrifuged at 500 g for 10 minutes, followed by PBS wash for two times. The cells were then treated with 75 μg RNAse A in 100 μl PBS and incubated at 37 oC for 1 hour. After incubation, the cells were collected by centrifuging at 500 g at 4 oC for 10 minutes, followed by another PBS wash. The cell pellet was re-suspended in 200 μl PBS, in addition of PI solution at a final concentration of 25 ng/μl. After staining, the cells were analyzed immediately using flow cytometer BD FACSCantoTM II (BD Biosciences, San Jose, CA) with an excitation wavelength at 488 nm and emission at 585 nm.

Instrument

- BD FACSCantoTM II (BD Biosciences, San Jose, CA)

Software

- FlowJo software, version 10

Cell population abundance

FACS was used to assess cell-cycle profile for purified skin keratinocytes.

Gating strategy

Gating was determined by using non-PI stained cells as negative control.

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.