Differential glucose requirement in skin homeostasis and injury identifies a therapeutic target for psoriasis

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Proliferating cells, compared with quiescent cells, are more dependent on glucose for their growth. Although glucose transport in keratinocytes is mediated largely by the Glut1 facilitative transporter, we found that keratinocyte-specific ablation of Glut1 did not compromise mouse skin development and homeostasis. Ex vivo metabolic profiling revealed altered sphingolipid, hexose, amino acid, and nucleotide metabolism in Glut1-deficient keratinocytes, thus suggesting metabolic adaptation. However, cultured Glut1-deficient keratinocytes displayed metabolic and oxidative stress and impaired proliferation. Similarly, Glut1 deficiency impaired in vivo keratinocyte proliferation and migration within wounded or UV-damaged mouse skin. Notably, both genetic and pharmacological Glut1 inactivation decreased hyperplasia in mouse models of psoriasis-like disease. Topical application of a Glut1 inhibitor also decreased inflammation in these models. Glut1 inhibition decreased the expression of pathology-associated genes in human psoriatic skin organoids. Thus, Glut1 is selectively required for injury- and inflammation-associated keratinocyte proliferation, and its inhibition offers a novel treatment strategy for psoriasis.

Glucose is a preferred bioenergetic and synthetic substrate for rapidly proliferating cells. However, recent studies have begun to highlight the diverse array of metabolic substrates that cells can use to promote growth. For example, although cancer cells consistently show a high capacity to utilize glucose as a metabolic substrate, they are capable of using diverse substrates (for example, amino acids, acetate, and lactate) for energy generation and anabolic growth in vivo. In some tissues, the preferential utilization of glycolysis has been linked to cell phenotype. For example, increased Glut1 expression and glucose utilization promote effector T cell function, whereas fatty acid oxidation (FAO) promotes regulatory CD4+ T cell and memory CD8+ T cell fates. In endothelial cells, glycolysis promotes vessel branching and migration, whereas FAO is required for proliferation. Thus, tissue functions are regulated in part through the controlled flow of metabolic substrates.

The facilitative glucose transporter (Glut) proteins regulate the availability of glucose in most tissues. The 13 Glut family members are regulated in a tissue- and stimulus-specific fashion, thus suggesting that they have important roles in shaping or maintaining cell function. Glut1, the most widely expressed facilitative glucose transporter, regulates basal glucose uptake in most tissues, including the basal cells of the epidermis. In agreement with a role in promoting keratinocyte proliferation, Glut1 expression is increased in wound healing, in psoriatic plaques, or after UV-induced hyperplasia. However, the precise role of Glut1 and glucose transport in regulating keratinocyte function has not been definitively characterized.

Here, we specifically deleted Glut1 in keratinocytes to determine how glucose metabolism affects epidermal development and function. In agreement with its known role in energy generation and biosynthesis, Glut1-deficient keratinocytes showed markedly impaired proliferation in vitro. Nonetheless, Glut1 deficiency had no effect on epidermal development or function in vivo. Gene expression analyses, metabolic profiling, and in vitro rescue experiments revealed that alternative hexoses, amino acid catabolism, and FAO all contributed to metabolic programs that allowed for epidermal function in the absence of Glut1. However, Glut1 deficiency impaired the physiological proliferation triggered by full-thickness wounds and UV irradiation, and genetic or chemical inhibition of Glut1 rendered the skin resistant to imiquimod- or IL-23-induced psoriasiform hyperplasia. Our results indicate that glucose metabolism is selectively essential for proliferating keratinocytes, highlighting a potential therapeutic target for pathological hyperproliferation.

Results

Glut1 is required for glucose uptake and proliferation. Although the expression of Glut1 in keratinocytes is well established, the regulation of Glut1 and the expression of additional glucose transporters in the skin has not been explored. Immunofluorescence revealed that Glut1 was primarily expressed in the more proliferative basal...
layers of both human and mouse skin (Supplementary Fig. 1a). In agreement with the reported role of Glut1 in cell proliferation, the expression of Glut1 was high in undifferentiated keratinocytes but decreased after either Ca^{2+}- or confluence-induced differentiation (Supplementary Fig. 1b,c). The mRNA expression of other facilitative (Glut1–Glut13 (official symbols Slc2a1–Slc2a13)) and sodium-dependent glucose transporters (Sglt1 and Sglt2 (official symbols Slc5a1 and Slc5a2)) in mouse epidermis, mouse and human primary keratinocytes, immortalized human keratinocytes (HEK001), and squamous cell cancers was assessed; only Glut1 was highly expressed (Supplementary Fig. 1d–h). Next, Glut1 was deleted in epidermal keratinocytes by crossing Glut1fl/fl mice to K14-Cre-expressing mice. In those mice (hereafter denoted K14.Glut1), compared with their Glut1fl/fl (hereafter denoted WT) littermates, Glut1 mRNA expression was >95% lower in both the epidermis and primary cultured keratinocytes. Western blotting and immunofluorescence confirmed that Glut1 was undetectable in the epidermis of K14.Glut1 mice (Fig. 1a,b and Supplementary Fig. 1j). Quantitative RT–PCR revealed no significant compensatory upregulation of any other glucose transporters in keratinocytes from K14.Glut1 mice (Fig. 1c). ¹H-labeled 2-deoxyglucose (2-DG) uptake assays in primary Glut1-deficient keratinocytes confirmed that Glut1 deletion blocked glucose transport. 2-DG uptake was ~96% lower in keratinocytes from K14.Glut1 mice compared with their WT littermates. The residual low level of glucose uptake in keratinocytes from K14.Glut1 mice was comparable to passive levels of transport, which occurred in the presence of chemical inhibitors of glucose transport (Fig. 1d).

Because Glut1 plays important roles in cell proliferation, we tested whether keratinocytes from K14.Glut1 mice showed defects in proliferation. The growth of primary Glut1-deficient keratinocytes was markedly impaired compared with that of WT keratinocytes, on the basis of crystal violet staining and cell numbers (Fig. 1e). GLUT1 was also essential for proliferation in human keratinocytes and primary cultured keratinocytes. Western blotting and immunofluorescence confirmed that, agreement with the results from the 2-DG uptake studies, keratinocytes from K14.Glut1 mice consumed less glucose and produced less lactate (~5%) than did their WT counterparts (Fig. 1f). ATP levels were also significantly lower in these keratinocytes (Fig. 1g). Decreased ATP levels can cause energetic stress and activate the S′-AMP-activated protein kinase. Indeed, downstream targets of this kinase, such as acetyl-CoA carboxylase 1 (ACC) showed increased phosphorylation in the knockout keratinocytes. In agreement with this energetic stress, phosphorylation of S6 ribosomal protein, a target of the mTOR kinase and a growth-promoting pathway, was inhibited in the keratinocytes from K14.Glut1 mice compared with WT controls (Fig. 1h).

Glut1 contributes to redox homeostasis. To discover additional pathways contributing to the impaired proliferation under glucose deficiency, we performed microarray analysis on keratinocytes from K14.Glut1 mice and their WT littermates (GSE102955; Fig. 2a and Supplementary Fig. 3a). In agreement with the significantly lower proliferative capacity of keratinocytes from Glut1-deficient mice, compared with their WT littermates, hierarchical clustering and gene ontology analyses revealed that the expression of genes related to cell-cycle progression and cell division was markedly impaired. Normal cells respond to oxidative stress by routing glucose through the pentose phosphate pathway (PPP) to regenerate NADPH,;22,23. In agreement with glucose’s role in the clearance of reactive oxygen species (ROS), transcripts related to redox homeostasis were among the genes with the most elevated expression in keratinocytes from K14.Glut1 compared with WT mice (Fig. 2a and Supplementary Fig. 3a). Twenty-four genes related to oxidative stress showed more than twofold-higher expression in Glut1-deficient keratinocytes than in WT keratinocytes (Fig. 2b and Supplementary Fig. 3b). Other oxidative-stress-response genes including Nqo1 and Hmox1 (also known as HO1) were significantly upregulated in Glut1-deficient keratinocytes compared with WT keratinocytes, both under basal growth conditions and when cells were treated with H₂O₂ or UV-B (Fig. 2c,d). Confirming the effect of Glut1 on cellular levels of oxidative stress, fluorescence measurements of the ROS indicator DCFDA revealed that keratinocytes from K14.Glut1 mice, compared with WT keratinocytes, showed significantly higher levels of ROS both under basal growth conditions and after treatment with H₂O₂ or UV-B (Fig. 2e). Glut1-deficient keratinocytes, compared with WT keratinocytes, were significantly more sensitive to H₂O₂- and UV-B-induced cell death, even after controlling for their slower growth (Fig. 2f). Supplementation with antioxidants (glutathione (GSH) or N-acetylcysteine (NAC)) rescued H₂O₂-induced toxicity in keratinocytes from K14.Glut1 mice (Fig. 2g). In primary human keratinocytes, the acute inhibition of GLUT1 through WZB117 also increased the sensitivity of the cells to H₂O₂ (Supplementary Fig. 2c) and increased the expression of redox-homeostasis genes (Supplementary Fig. 2d). Both genetic and chemical inhibition of glucose transport lowered levels of NADPH, as compared with those after treatment with their respective controls (Fig. 2h and Supplementary Fig. 2e,f), thus suggesting that decreased flux through the PPP contributed to the observed defects in redox homeostasis. Mitochondrial superoxide staining further revealed that acute (but not chronic) chemical inhibition of glucose transport, as compared with control vehicle treatment, increased mitochondrial oxidative stress (Supplementary Fig. 2g), thus suggesting that mitochondria may be an additional source of oxidative stress in keratinocytes deprived of glucose. Despite Nrf2’s established role in mediating the response to oxidative stress, the total and nuclear levels of Nrf2 were not higher in keratinocytes from K14.Glut1 mice (Supplementary Fig. 4a,b). Thus, Glut1 is required for redox homeostasis in keratinocytes at least in part through its roles in regenerating NADPH.

Glut1 is not essential for normal skin development. Given the striking effect of Glut1 deletion on keratinocyte growth, we assessed the in vivo effects of Glut1 deletion. Notably, K14.Glut1 mice appeared normal: we observed no abnormalities in the skin of young or adult K14.Glut1 mice relative to their WT littermates (Supplementary Fig. 5a). Both male and female K14.Glut1 mice showed normal skin histology at 1 week and 2 months (Fig. 3a). The expression and localization of epidermal keratins (K10 and K14) (Fig. 3b and Supplementary Fig. 5b–d), loricrin (Supplementary Fig. 5e), and differentiation and adhesion genes (Fig. 3c) showed no differences between WT and K14.Glut1 littermates, thus suggesting normal epidermal differentiation.

Given previous reports that glycosylated ceramides and UDP–glucose are essential intermediates for epidermal ceramide maturation, lamellar body formation, and stratum corneum development, we performed an analysis of epidermal lipids. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) revealed that the epidermis of K14.Glut1 mice, compared with WT, showed unchanged levels of phospholipids, lower levels of free ceramides and sphingoid bases, and higher levels of sphingomyelins (Fig. 3d, Supplementary Fig. 5f and Supplementary Dataset 1). Notably, hexosylceramide levels were significantly lower in the keratinocytes from K14.Glut1 mice but not in the epidermis of K14.Glut1 neonatal mice, as compared with WT levels (Fig. 3e). Moreover, K14.Glut1 and WT mice showed no significant differences in weight either as pups or adults (Fig. 3f). Compared with WT embryos, K14.Glut1 embryos showed both normal toluidine blue–exclusion assay results (Fig. 3g) and no defects in transepidermal water loss, as assessed by a weight
Fig. 1 | Primary keratinocytes showed impaired proliferation after Glut1 deletion. a. Glut1 mRNA (n = 4 mice per genotype) and protein levels in the epidermis and primary keratinocytes harvested from 1-week-old WT, K14.Glut1<sup>fl/fl</sup>, and K14.Glut1 mice. HSP90, loading control. b. Immunostaining of Glut1 in skin from 1-week-old mice. Glut1 staining is absent in epidermis from K14.Glut1 mice. Arrows indicate persistence of Glut1 staining in the dermis (for example, dermal endothelial cells). DAPI, nuclear stain. Similar results were obtained in three independent experiments. Scale bar, 10 μm. c. mRNA abundance for the indicated Glut and SGLT family members in primary cultured keratinocytes from WT, K14.Glut1<sup>fl/fl</sup>, and K14.Glut1 mice (n = 4 mice per genotype). d. 2-Deoxy-D-glucose uptake in primary cultured keratinocytes obtained from 1-week-old WT, K14.Glut1<sup>fl/fl</sup>, and K14.Glut1 mice with or without glucose-inhibitor pretreatment. Similar results were obtained in uptake assays from keratinocytes from three independent mice. CPM, counts per minute. e. Growth rate, as assessed by crystal violet staining and relative cell number for primary keratinocytes from WT, K14.Glut1<sup>fl/fl</sup>, and K14.Glut1 mice. Identical numbers of cells were seeded in triplicate wells in six-well plates at day 0. For crystal violet staining, cells were stained 3 d after seeding, and similar results were obtained for three keratinocyte preparations. Keratinocytes from K14.Glut1 mice showed significantly less growth than did keratinocytes from WT controls at days 1 and 2 after plating. f. Glucose consumption and lactate production over 12 h, as measured in the medium of primary cultured WT and K14.Glut1 (n = 3 mice per genotype) keratinocytes. g. ATP levels (fluorescence; AU, arbitrary units) determined in primary keratinocytes from WT and K14.Glut1 (n = 3 mice per genotype) and normalized to protein levels. h. Western blot analysis of the expression of p-ACC Ser79, ACC, p-S6 Ser240/244, CoxIV, and Glut1 in primary keratinocytes obtained from WT, Glut1<sup>fl/fl</sup> mice, and K14.Glut1 mice. Data are shown as mean ± s.d. P values were calculated with two-tailed t test. Results were confirmed in at least two independent experiments.

Metabolic compensation for Glut1 deficiency. To gain insight into how K14.Glut1 mice adapted to the loss of glucose uptake, neonatal epidermis was harvested for analysis of intracellular metabolites by LC–MS/MS (Supplementary Dataset 2). Principal component analysis showed different metabolic profiles for the epidermis from K14.Glut1 mice and WT mice (Supplementary Fig. 6a). Quantitative pathway enrichment analysis of epidermal metabolites from Glut1-deficient mice, compared with their littermate controls, revealed a significant enrichment in several expected pathways including nucleotide sugar metabolism, the tricarboxylic acid (TCA) cycle, and the PPP (Supplementary Fig. 6b). Significantly lower levels of numerous metabolites, including glucose-6-phosphate (G6P), lactate, aconitate, malate, and fumarate, confirmed the expected impairments in glycolysis and the TCA cycle (Fig. 4a). Metabolomics also revealed significant changes in amino acid metabolism and the urea cycle, including changes in glutamine, cystathionine, citrulline, carbamoyl phosphate, and acetylornithine (Fig. 4b). In agreement with the increased catalysis of amino acids, the expression of numerous amino acid transporters was significantly higher in keratinocytes from K14.Glut1 mice compared with WT controls (Supplementary Fig. 6c). Finally, Glut1 deletion also affected nucleotide metabolism, as evidenced by lower levels of UMP and dAMP and higher levels of allantoin in the epidermis in K14.Glut1 mice compared with WT controls (Fig. 4c).

time-course analysis of newborn mice (Fig. 3h). Finally, in contrast to the previously described defects in redox homeostasis, Glut1-deficient epidermis showed no marked alterations in the expression of redox-homeostasis genes or evidence of energetic stress, as determined by western blot analyses (Supplementary Fig. 5g,h). Thus, although keratinocytes from K14.Glut1 mice showed marked alterations in lipid metabolism, redox homeostasis, and energetic stress in vitro, these defects were rescued in vivo, thus allowing for normal skin development.
Fig. 2 | Glut1 is required for proliferation and redox homeostasis in primary keratinocytes. a, Hierarchical clustering of transcriptional profiles from keratinocytes from WT and K14.Glut1 mice. Labels identify gene clusters showing enrichment gene ontology analyses (n = 8 mice per genotype). FC, fold change. b, RT–PCR measurements showing significant upregulation of the indicated redox genes in keratinocytes from K14.Glut1 and WT mice (n = 4 mice per genotype). c, d, Nqo1 (c) and Hmox1 (d) mRNA abundance in the indicated keratinocytes with or without H2O2 (100 μM) or UV-B (10 mJ/cm²) stimulation for 6 h (n = 3 mice per genotype). e, FACS analysis of the fluorescence after DCFDA staining of the indicated keratinocytes with and without H2O2 (100 μM, 30 min) and UV-B (10 mJ/cm², 30 min) stimulation (n = 3 independent mice per genotype). NS, nonsignificant. f, Keratinocytes were incubated with different concentrations of H2O2 for 24 h. Survival is plotted as the ratio of the number of surviving cells to the number of plated cells per genotype without H2O2 treatment (n = 3 mice per group). g, Keratinocytes were pretreated with GSH or NAC, then incubated with H2O2 for 24 h. Survival is plotted relative to that of untreated controls (n = 3 mice). h, Keratinocytes from K14.Glut1 mice, showing significantly lower levels of NADPH (n = 4 mice per genotype). Data are shown as mean ± s.d. P values (indicated above relevant comparisons) were calculated with two-tailed t test (b,h), two-way analysis of variance (ANOVA) with Tukey’s tests (c–e), and one-way ANOVA with Dunnett tests (g). Results were confirmed in at least 2 independent experiments.

Metabolic profiling highlighted changes in galactose metabolism in the epidermis of K14.Glut1 mice. Intracellular galactose-1-phosphate was maintained at similar levels as G6P and fructose-6-phosphate and was depleted to similar levels as G6P and fructose-6-phosphate in the epidermis of K14.Glut1 mice, thus suggesting that it was actively metabolized in the epidermis (Fig. 4a). Moreover, the keratinocytes expressed Glut5, SGLT1 (Supplementary Fig. 1d,e), and ketohexokinase, which should have allowed for fructose metabolism to occur. Therefore, we tested whether galactose and fructose could rescue the growth of Glut1-deficient keratinocytes. In keratinocytes from Glut1-deficient mice compared with WT mice, fructose and galactose significantly improved keratinocyte growth (Fig. 4e,f and Supplementary Fig. 7a,b), ATP levels (Fig. 4g), expression of redox-homeostasis genes (Fig. 4h), and resistance to H2O2 (Fig. 4i). Fructose almost fully rescued the growth and oxidative-stress defects of keratinocytes from K14.Glut1 mice, whereas galactose was less effective. Because FAO contributes reducing equivalents and acetyl-CoA in endothelial cell metabolism14, we tested whether mixtures of oleate and palmitate could rescue the growth of Glut1-deficient keratinocytes. The addition of up to 10 μM of a fatty acid mixture partially rescued the growth of keratinocytes from K14.Glut1 mice and increased ATP production in both Glut1-deficient and WT keratinocytes (Fig. 4j,k). In contrast, neither a myriad of downstream metabolites and antioxidants (including pyruvate, lactate, amino acids, GSH, NAC, glutamine, or ribose), supplemented individually or in combination (Supplementary Fig. 7c–h), nor growth under hypoxic conditions (Supplementary Fig. 7i) was able to rescue Glut1-deficient keratinocyte growth in vitro. In summary, keratinocytes can utilize galactose, fructose, and fatty acids as metabolic substrates to promote growth in the absence of Glut1 (Supplementary Fig. 7j).

Glut1 is required for stress responses in vivo. We next tested whether Glut1 was necessary for the epidermal responses to physiological stress. One critical function of human skin is protection from UV irradiation. Chronic irradiation has been reported to promote both Glut1 expression and acanthosis15. We shaved and
UV-B-irradiated K14.Glut1 mice and WT littermates to determine the role of glucose transport in the acute and delayed response to UV-B (Supplementary Fig. 8a). There were no obvious macroscopic or histologic differences in the skin of K14.Glut1 mice compared with their WT littermates 24 h after UV-B treatment (Fig. 5a and Supplementary Fig. 8a,b). UV-B induced similar significant increases in skin thickness in both WT and K14.Glut1 mice (Supplementary Fig. 8c). TUNEL staining for apoptotic keratinocytes also showed no differences in the skin between WT and K14.Glut1 mice (Supplementary Fig. 8d,e). By 5 d after UV-B treatment, the WT mice had largely recovered and showed regular acanthosis, whereas many areas of skin from the K14.Glut1 mice showed areas of abnormal stratification with areas of keratinocyte necrosis (Fig. 5a and Supplementary Fig. 8b). Although nonirradiated epidermis did not show differences in redox-homeostasis genes, 6 h after UV-B treatment, epidermis from Glut1-deficient mice, compared with WT controls, showed a modest but significant upregulation of oxidative-stress-response genes. These changes were no longer significant 24 h after UV-B treatment (Fig. 5b and Supplementary Figs. 5g and 8f). Significantly fewer Ki-67+ cells were present in the epidermis of K14.Glut1 mice compared with WT controls (Fig. 5c,d). These defects in proliferation were corroborated by elevated staining of phosphorylated (p-) ACC Ser79 and decreased staining of p-S6 Ser240/244 in the skin in Glut1-deficient mice compared with WT controls after UV-B treatment (Supplementary Fig. 8e). Keratinocytes secrete inflammatory cytokines in response to UV-B17, and these cytokines contribute to the physiological response to irradiation. UVB-induced inflammation,
as assessed by expression of the cytokines IL-1β, TNFα, IL-6, and IFNγ, revealed no significant differences between littermates (Supplementary Fig. 8h). In summary, compared with WT mice, K14.Glut1 mice showed impairments in redox homeostasis, proliferation, and recovery after UV-B treatment.

The epidermis also undergoes rapid proliferation in response to wounds. To understand the role of Glut1 in wound repair, we assessed the recovery of mice from splinted 3-mm excisional wounds (Supplementary Fig. 9a). Reepithelialization was assessed after 5.5 and 8 d. Both K14.Glut1 and WT mice exhibited lymphohistiocytic infiltrates around the wound edge (Supplementary Fig. 9b). However, healing in the K14.Glut1 mice, compared with their WT littermates, was significantly impaired at both time points (Fig. 5e,f). Specifically, by day 8, the WT mice had largely completed wound healing, whereas all the K14.Glut1 mice still exhibited persistent epidermal defects (Fig. 5e and Supplementary Fig. 9b). Keratinocyte migration and proliferation both contribute to wound healing28. To study cell migration in Glut1-deficient keratinocytes, we analyzed keratinocyte migration in scratch assays through time-lapse microscopy. Keratinocytes from K14.Glut1 mice, compared

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Glucose-transport inhibition ameliorates psoriasis models. Although keratinocyte hyperproliferation can be triggered as a protective response against physiological insult, it can also be triggered pathologically. Psoriasis is a chronic cytokine-driven, inflammatory skin disease characterized by hyperplasia and abnormal differentiation of the epidermis that presents as thickened and scaly plaques. After confirming that GLUT1 transcription and expression were elevated in lesional biopsies from people with psoriasis (Supplementary Fig. 10a–c), we tested whether Glut1 deletion might affect the development of pathological hyperplasia in diseases such as psoriasis. We established psoriasiform hyperplasia in WT and K14.Glut1 mice through two models: topical application of imiquimod and intra-dermal injection of IL-23 (ref. 31). The skin of WT mice treated with imiquimod showed upregulated Glut1 expression (Supplementary Fig. 10a,d,e) and acanthosis (Supplementary Fig. 10a). In contrast, the epidermis of K14.Glut1 mice was markedly protected from imiquimod-induced psoriasiform hyperplasia (Fig. 6a and Supplementary Fig. 10g). To extend the translational relevance of these findings, we tested whether the topical inhibition of glucose transport might also prevent the development of psoriasiform hyperplasia (Supplementary Fig. 10f). In both mouse models of psoriasiform hyperplasia, treatment with the topical GLUT inhibitor WZB117, compared with vehicle control, decreased scale (Fig. 6a and Supplementary Fig. 10g,h) and significantly inhibited skin thickening (Fig. 6b,c and Supplementary...
Fig. 6 | Genetic or topical inhibition of glucose transport decreases psoriasiform hyperplasia. **a**, *K14.Glut1* mice, compared with WT littermates, have less scale and erythema after treatment with imiquimod. Similar results were obtained in 3 independent experiments. Scale bar, 1 cm. **b**, Histologic sections showing that genetic (*K14.Glut1*) and chemical (WZB117) inhibition of glucose transport both decrease the acanthosis and parakeratosis induced by both imiquimod and IL-23 injections. Similar results were obtained in 4 independent experiments. Scale bar, 100 μm. **c**, *K14.Glut1* and WZB117-treated skin showed significantly less acanthosis after both imiquimod- and IL-23-induced psoriasiform dermatitis. Acanthosis was scored blindly as the number of nucleated keratinocytes (n = 6 mice per group). **d**, Ki-67 staining (5 d) after imiquimod- or IL-23-induced psoriasiform dermatitis treatment, showing substantially less proliferation in *K14.Glut1* and WZB117-treated mice. Similar results were obtained in 3 independent experiments. **e**, The percentage of Ki-67+ cells in epidermal basal cells (per 50 cells), quantified in skin sections from control, *K14.Glut1*, and WZB117-treated mice (n = 6 mice per group). **f**, Staining of skin sections of WT, *K14.Glut1*, or WZB117-treated mice were treated with imiquimod for 6 d, and (n = 4 mice per group). Treatment with WZB117 significantly decreased the number of leukocytes (CD45+), neutrophils (Gr-1+), macrophages (F4/80+), and T cells (CD4+). **g**, RT–PCR analysis of psoriatic biomarkers in harvested epidermis of human psoriatic skin organoids treated with vehicle or WZB117 (6 or 30 mg/ml) for 24 h (n = 3 organoids). Box shows twenty-fifth to seventy-fifth percentiles; whiskers show minimum to maximum; crosses show means; and lines show medians. Bars show mean ± s.d. P values (indicated above relevant comparisons) were calculated with one-way ANOVA with Holm-Sidak tests (**c**, **e–g**). Results were confirmed in at least 2 independent experiments.

Fig. 10h). The skin of *K14.Glut1* mice and WZB117-treated WT mice showed lower Ki-67 staining than that of vehicle-treated WT controls (Fig. 6d,e). Western blotting confirmed that the skin of both *Glut1*-deficient mice and WZB117-treated mice showed elevated p-ACC and lower p-S6 Ser240/Ser244 levels, results consistent with energetic stress (Supplementary Fig. 10l).
In addition to inducing keratinocyte proliferation, imiquimod also induces the accumulation of leukocytes in the skin, including neutrophils, macrophages, dendritic cells, and T cells. We examined the effect of inhibition of glucose transport on inflammation through immunohistochemistry (IHC) staining. Topically applied WZB117 significantly decreased the numbers of leukocytes (CD45^+), neutrophils (Gr-1^+), macrophages (F4/80^+), and T cells (CD4^+), but not dendritic cells (CD11c), localized to the skin after treatment with imiquimod compared with vehicle control. In contrast, the skin of K14.Glut1 mice showed significant decreases in only T cell number (CD4^+) after imiquimod treatment (Fig. 6f and Supplementary Fig. 11a–f). Similarly, the mRNA levels of several cytokines (CCL3, CXCL3, and IL-1β) that are upregulated in imiquimod-induced psoriasiform dermatitis showed significant decreases in WT mice topically treated with WZB117; however, the levels in the K14.Glut1 mice were not significantly different from those in vehicle-control-treated WT mice (Supplementary Fig. 11g). Finally, we applied WZB117 topically to human psoriatic skin organoids. WZB117 inhibited the expression of several psoriatic biomarkers (CXL3, P13 (also known as ELAFIN), DEFB4A (also known as HBD-2), S100A7, and S100A8) in a dose-dependent fashion (Fig. 6g). Thus, whereas genetic Glut1 deletion rescued only the epidermal acanthosis and proliferation, the topical, chemical inhibition of glucose transport rescued keratinocyte proliferation and also prevented inflammation in animal and organoid models of psoriasiform inflammation.

Discussion

The epidermis is a self-renewing organ that protects the body against environmental insults and excess water loss. How glucose uptake and metabolism contribute to these essential processes has not been explored in vivo. Through the deletion of the Glut1 transporter in keratinocytes, we found that glucose uptake is critical for rapid keratinocyte proliferation and is an efficient response to physiologically relevant stressors including full-thickness wounds and UV-B irradiation. Despite evidence of stressed energy metabolism and oxidative stress in vitro, K14.Glut1 mice showed normal epidermal development. In keratinocytes, in contrast to many other tissues, Glut1 is the only substantially expressed glucose transporter, and its deletion largely abolished glucose transport. Thus, our metabolic analyses of Glut1-deficient keratinocytes have broader implications regarding how other tissues and cancer cells might adapt to a general block of glucose metabolism.

Because glucose contributes to several metabolic pathways, cells induced multiple compensatory pathways in its absence. Our findings highlight the importance of the PPP in the skin, as has previously been demonstrated in vitro. Transcriptional upregulation of many oxidative-stress-response genes was observed in the Glut1-deficient keratinocytes. Under conditions of decreased hexose availability, metabolic processes involving central carbon metabolism, including glycolysis, the TCA cycle, and nucleotide synthesis, were suppressed. Transcriptional and metabolic analyses suggested that alterations in amino acid metabolism might help to maintain these biosynthetic cycles. Specifically, the pattern of alterations in amino acid metabolism may be explained by increased catabolism of specific amino acids to generate ketocoids (for example, α-ketoglutarate from glutamine and α-ketobutyrate from cystathionine) to compensate for the loss of glucose. In addition to alterations in amino acid metabolism, we also found that fatty acids partially rescued proliferative defects in Glut1-deficient keratinocytes. In vitro, alternative hexoses, especially fructose, rescued the proliferative defects of Glut1 deficiency. Despite the low circulating levels of these hexoses, galactose was detected intracellularly in both normal and Glut1-deficient epidermis, thus suggesting that these hexoses may contribute to normal skin development in the absence of glucose. Galactose rescued growth less efficiently than did fructose, a result reflecting either inefficient transport of galactose by non-Glut1 facilitative glucose transporters or limitations in the efficiency of the Leloir pathway in keratinocytes. However, supplementation with other downstream metabolites did not rescue the growth of keratinocytes from K14.Glut1 mice. We speculate that the inherent limitations of in vitro culture systems and the central role of glucose in diverse metabolic and signaling pathways may have limited our ability to rescue the growth of Glut1-deficient keratinocytes.

Although the generation of mitochondria-derived ROS is necessary for skin differentiation, the increased oxidative stress caused by loss of glucose transport does not affect skin differentiation. The glycosylation of both proteins and lipids plays an important biochemical role in the skin. Specifically, glycosylated ceramides have been found to be essential for the proper formation of an intact stratum corneum, because the deletion of glucosylceramide synthase (Uggs gene) results in a disruption of normal ceramide metabolism, aberrant stratum corneum development, and perinatal lethality. Despite defects in hexosylceramide synthesis in vitro, we found the skin barrier to be intact, thus suggesting that the epidermis is able to maintain sufficient levels of ceramides for the maturation of epidermal ceramides even in the absence of Glut1.

Because glucose uptake is required for proliferating keratinocytes but not for normal skin development and function, our findings suggest that inhibiting glucose transport may be a promising therapy for skin diseases. Antimetabolites that target the heightened biosynthetic requirements of proliferating cells—including methotrexate, 5-fluorouracil, and mycophenolic acid—are some of the most effective treatments for inflammatory and neoplastic diseases. Although most antimetabolite therapies have been discovered fortuitously, our analysis of Glut1 uncovered glucose transport as a potential target. Psoriasis is characterized by excessive cytokine-driven epidermal hyperplasia. Psoriatic lesions have heightened requirements for glucose uptake and metabolism, as evidenced by increased Glut1 expression and positron emission tomography scans. Metabolomic studies have demonstrated elevated serum levels of some amino acids in patients with psoriasis. Our metabolic analysis of the epidermis of Glut1-deficient mice revealed decreases in a similar set of amino acids. Levels of sphingoid bases were elevated in the serum and skin of patients, in agreement with our finding that levels of these lipids were lower in the skin of Glut1-deficient mice than WT mice. Comparing the metabolomics from patients and from Glut1-deficient mice suggested that inhibiting glucose transport may affect metabolic pathways critical to the development of psoriasis. In agreement with this prediction, Glut1-deficient mice were protected against imiquimod-induced psoriasiform hyperplasia. Although the systemic administration of a glucose-transport inhibitor has been predicted to cause neurologic sequelae, hyperglycemia, and lipodystrophy, these toxicities might be avoided through the topical use of glucose-transport inhibitors. Indeed, the topical application of a Glut1 inhibitor was also effective in protecting mice against imiquimod-induced psoriasiform hyperplasia. Similarly to the deletion of Glut1 in keratinocytes, chemical inhibition of Glut1 inhibited epidermal proliferation and acanthosis. Topical transport inhibitors had the added feature of suppressing inflammatory infiltrates and cytokine secretion in animal models of psoriasis and human psoriatic skin organoids, respectively. In agreement with previously reported roles of Glut1 and glucose metabolism in T lymphocyte activation, we speculate that the topical application of WZB117 might also inhibit glucose uptake in other cell types in the skin, including infiltrating lymphocytes, and thereby limit inflammation in vivo. In summary, our study provides insight into the metabolic reprogramming that allows cells to survive in the absence of glucose metabolism, highlights the role of glucose transport after physiological stressors, and identifies glucose transport as a viable target in hyperproliferative skin diseases.
Methods
Methods, including statements of data availability and any associated codes and references, are available at https://doi.org/10.1038/s41591-018-0003-0.

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Author contributions
Z. Zhang, R.J.D., and R.C.W. designed the experiments. Z. Zhang, E.E.L., J.Z., M.M., and R.C.W. performed experiments. E.D.A. provided Glut1<sup>fl/fl</sup> mice. A.P.S. provided SCCT8 squamous cell carcinoma cells. B.F.C. enrolled patients. Z. Zhang, Z. Zi, E.E.L., J.Z., D.C.C., M.M., G.A.H., T.V., J.C.R., P.E.S., R.J.D., and R.C.W. analyzed data; R.C.W. and Z. Zhang wrote the manuscript, to which all authors contributed.

Competing interests
The authors declare no competing interests.

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Methods

All animal procedures were performed under protocols approved by the University of Texas Southwestern Medical Center Institutional Animal Care and Use Committee (IACUC), animal protocol number 2015-101166. All efforts were made to reduce suffering and to minimize distress, refinement and reduction guidelines. To minimize their discomfort, mice were anesthetized with a ketamine/xylazine cocktail before being killed. Patients were recruited from the University of Texas Southwestern Medical Center outpatient clinics and provided written informed consent to participate in a research study. The study was approved by the UTSW Institutional Review Board, IRB protocol number STU 08210-241.

Animal models. Glut1<sup>fl/fl</sup> mice were provided by E. D. A. and were mated to C57BL/6J (Jackson Laboratory) for two generations to remove background effects. The offspring were genotyped by PCR and used in all experiments. Male K14-Cre mice were backcrossed to C57BL/6J mice in order to obtain a pure background.

Animal care and treatments.

HEK<sub>001</sub> (ATCC CRL-2404) cells were cultured in KSF medium (Invitrogen, 37010022). SCCT8 cells (provided by A.P.S.) were cultured in DMEM/F-12 (Gibco, 11320082) supplemented with Glutamax (Gibco, 35050061), 10% FBS, 0.1 mg/ml hydrocortisone (Sigma, H0888), 10 mg/ml EGF (Invitrogen, 10450-013), 100 U/ml penicillin, 100 µg/ml streptomycin, 0.5 µg/ml bovine pituitary extract (Thermo Fisher) in PBS:0.1% BSA. The appearance of the skin was monitored daily, and tissues were harvested, typically after 5 d of treatment. For W2B117 treatment, WT mice were randomly assigned to receive either W2B117 (100 µl of 1 mg/ml) or acetone vehicle control. The WZB treatment was applied topically immediately before imiquimod treatment or IL-23 injection.

Histological analyses and immunohistochemistry.

For IHC, tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Sections (5 µm) were deparaffinized, stained with hematoxylin and eosin (Sigma, H1109) or immunostained according to standard protocols or the manufacturer’s instructions. Detection of IHC signal was performed with a Vectastain Elite ABC kit (Vector Laboratories) and DAB substrate kit for peroxidase (Vector Laboratories), and this was followed by hematoxylin counterstaining (Vector Laboratories). For scoring of leukocytes, four sections from at least three mice per group from at least two independent experiments were examined, and photomicrographs were taken at representative 40× magnification fields. Positive cells in each photomicrograph were quantified. Immunofluorescence of Glut1 and K14, after incubation with primary antibodies, were washed and incubated with Alexa Fluor 488 goat anti-rabbit or Alexa Fluor 488 goat anti-mouse secondary antibodies (Life Technologies, A11010 and A11011). In room temperature for 1 h, then washed and sealed with Prolong Gold Antifade reagent with DAPI (Life Technologies, P36935). TUNEL staining (Thermo Fisher, C10617) was performed according to the manufacturer’s protocol.

RNA extraction and qRT–PCR.

Cells or tissues were lysed in Trizol reagent (Thermo Fisher, 15596024), and mRNA was extracted according to standard protocols and reverse transcribed with a Thermoscript RT system (Invitrogen, 11164016) according to the manufacturer’s instructions.

In vitro wound-healing assays.

For the wound-healing assays, cells were plated at a several densities ranging from 0.5 × 10<sup>3</sup> to 1 × 10<sup>5</sup> cells per well in a six-well plate (three wells per cell line) and cultured for 24 h under standard culture conditions. On the day of the assay, control and knockout cells showing comparable densities were selected for assessment. Three parallel lines per well were ‘drawn’ with gentle pressure with a sterile 1,000-µl pipette tip. Cells were washed twice with PBS to remove cell debris before the addition of prewarmed standard culture medium. The plate was then placed into the temperature- and CO<sub>2</sub>-controlled chamber (G250; Nano-Imag, a Nikon Ti Eclipse microscope with perfect focus and stage and a Zyla 4.2 sCMOS camera (Andor) driven by the NIS Elements V4.13 software package. Wound closure (one region of interest per scratch) was recorded through phase contrast with a 10× air objective at a frame rate of one image per 10 min for 24 h. For quantification in ImageJ, the wound area was measured manually in the first and last frame of each dataset (eight per cell line), and the results are expressed as efficiency of wound closure (100 − ([area of first frame] − [area of last frame])).

[1]H<sub>2</sub>-deoxyglucose uptake assay. 2-DG uptake assays were performed as previously described<sup>61</sup>. Primary mouse or human keratinocytes (50,000 cells) were seeded in triplicate in 12-well plates overnight, washed twice with PBS (Sigma, D5762) or Hank’s balanced salt solution (in buffer or serum-free DMEM/F12 medium (Thermo, 11320033 for SCCT8 cells) for 2 h, washed twice with KRHP buffer, incubated in 0.45 mL KRHP buffer per well, and starved for 30 min. For glucose-transporter inhibitor, cytochalasin B (10 µM, Sigma, C6762) and phloretin (100 µM, Sigma, P7912) were added to the KRHP medium for another 5 min. Untreated control was treated with 0.5% DMSO. WZB treatment was applied to keratinocytes at 100 µM in 0.5% DMSO, Phloretin treatment was applied to keratinocytes at 100 µM in 0.5% DMSO. WZB or phloretin was added to the KRHP medium 5 min before UV-B lamp irradiation. The dose of the UV-B lamp was measured with an IL-700 Research Radiometer (International Light Technology). Cells or mice were placed at least 20 cm from the light source. For the excisional-wound splitting model, 2-month-old mice were shaved and chemically depilated. The dorsal skin of each mouse, at the midline between the spinous and nuchal region, was excised with a 3-mm biopsy punch (Millex) to the level of the panniculus carnosus. A sterile circular silicone splint was attached to the skin with Krazy glue, then secured with 5-0 Prolene sutures. A topical antibiotic (bacitracin, polymyxin B, and neomycin) ointment was applied, and wounds were dressed with Tegaderm transparent dressing daily. The wound size was measured daily and the area was plotted as initial size, and for the imiquimod-induced psoriatic hyperplasia model, 2-month-old mice were shaved and chemically depilated. The shaved dorsal-skin samples were treated topically with 50 µg of Aldara cream (5% imiquimod) (Aldara, 3M Pharmaceuticals) daily. For the intradermal IL-23-induced psoriatic hyperplasia model, shaved and depleted 2-month-old mice were injected daily at a labeled location on the central back with 1 µg intradermal IL-23 (Thermo Fisher) in PBS:0.1% BSA. The appearance of the skin was monitored daily, and tissues were harvested, typically after 5 d of treatment. For WZB117 treatment, WT mice were randomly assigned to receive either WZB117 (100 µl of 1 mg/ml) or acetone vehicle control. WZB treatment was applied topically immediately before imiquimod treatment or IL-23 injection.
the internal control and are presented as relative expression levels. All primers for qRT–PCR are described in Supplementary Table 1.

Cytometry and proliferation measurements. For basal levels of oxidative stress, cells were stained with 5 µM DCFDA (Thermo, D-399) for 30 min, washed, trypsinized, collected on ice, centrifuged at 1,500 r.p.m. for 4 min, washed once with FACS buffer (DPBS with 2% FBS (Sigma, F2442) and 0.2 mM EDTA (Ambion, AM9260G)), and then resuspended in FACS buffer for analysis (FACScalibur, BD). For oxidative stress induced by H2O2, cells were supplemented with 5 µM DCFDA, then incubated in the indicated amount of H2O2, and harvested after 30 min of incubation. For oxidative stress induced by UV-B irradiation, cells were incubated with 5 µM DCFDA for 10 min, the medium was transferred to new tubes, the cells were washed once with PBS and irradiated with the indicated doses, the medium was returned to the cells and incubated for another 20 min, and the cells were harvested for FACS analysis. For cultured-cell proliferation, cells in equal numbers were seeded, harvested at the indicated times, and then counted in the presence of trypan blue (Bio-Rad, 145-0013). For staining, cells were stained with 0.25% crystal violet (Sigma, C0775) in 6.25% ethanol (Pharmco-AAPER, 111000200) for 30 min at room temperature, washed three times with ddH2O, dried at room temperature, and photographed. For MTT assays, cells were incubated for 2 h with 0.5 mg/ml MTT (Thermo, M6494) dissolved in DMEM (Sigma D4540), and the absorbance was measured at 540 nm. For the keratinocyte proliferation assays in tissues, the tissues were stained for Ki-67 as described in the histological analyses section above.

ATP measurements. Cellular ATP content was measured with a CellTiter-Glo luminescence assay kit (Promega, G7570). Briefly, cells were seeded in at least triplicate wells at a density of 10,000 cells/well in two 96-well plates and allowed to attach overnight. One plate of cells was used for measuring the concentrations of protein through the BCA method. Another plate of cells was placed at room temperature for 30 min. Then 100 µl of lysis buffer with CellTiter-Glo substrate was added and mixed well, and cells were placed on an orbital shaker for 15 min at room temperature. Cellular ATP levels were measured with a luminescence plate reader (BioTek). A standard curve of ATP was made to ensure that the concentrations of cellular ATP were within the linear range.

Lipid analyses. Epidermis from WT (n = 4) and K14.Glut1 (n = 4) mice was harvested for lipid analysis. Sphingolipid levels were quantified with LC–MS/MS methodology1. Briefly, flash-frozen epidermis pieces were homogenized in 500 µl of ice-cold HPLC water with a sonic dismembrator probe, and a 50-µl aliquot was reserved for protein determination. Immediately afterward, the aqueous homogenate was quenched by addition to 2 ml of organic extraction solvent (isopropanol/ethyl acetate, 15:85 (vol/vol) in a borosilicate glass culture tube). The original sample tube was rinsed twice with 500 µl of cold HPLC water, and the aqueous emulsions were added to the organic extraction solvent. 20 µl of internal standard solution was added (Avanti Polar Lipids, AL Ceramide/Sphingoid Internal Standard Mixture II diluted 1:10 in ethanol). The mixture was vortexed and sonicated in an ultrasonic bath for 10 min at 40 °C. Then the samples were allowed to reach room temperature. Two-phase liquid extraction was performed, the supernatant was transferred to a new tube, and the pellet was reextracted. Supernatants were combined and evaporated under nitrogen. The dried residue was reconstituted in 2 ml of Folch solution, and 500 µl was transferred to a new tube and reserved for organic phosphate determination. Both organic fractions were dried under nitrogen and stored at −80 °C until analysis. Sphingolipid levels were quantified through LC–MS/MS with a Nexera X2 UHPLC system coupled to a Shimadzu LCMS-8050 triple-quadrupole mass spectrometer operating with a dual-ion-source in electrospray positive mode. Dried lipid extracts were reconstituted in 200 µl of HPLC solvent (methanol/formic acid 99:1 (vol/vol) containing 5 mM ammonium formate) for LC–MS/MS analysis. Lipid separation was achieved on a 2.1 (i.d.) × 150-mm Kinetex C8, 2.6-micron core-shell particle (Phenomenex) column. Sphingolipid species were identified on the basis of exact mass and fragmentation patterns and verified by using lipid standards. The concentration of each metabolite was determined according to calibration curves by using the peak-area ratio of the analyte versus the corresponding internal standard. Calibration curves were generated by using serial dilutions of each target analyte. Sphingolipid standards were purchased from Avanti Polar Lipids. For total phosphorus determination, total phosphorus content in the organic extracts was determined as previously described23.

Metabolite profiling. Epidermis was harvested from the dorsal skin of WT and K14.Glut1 mice. Each epidermal sample was from one mouse, and eight pairs of samples were used for metabolomic analysis as previously described23. The harvested epidermis was snap frozen, and metabolites were extracted with 80% ice-cold methanol. Metabolite profiling was performed with LC–MS/MS. The peak area for each detected metabolite was normalized to the total ion count. The preprocessed datasets were mean-centered and unit-variance scaled. Principal component analysis and hierarchical clustering of metabolites in different samples were performed in MetaboAnalyst 3.0.

Microarray analyses. Primary keratinocytes derived from WT (n = 8) and K14.Glut1 (n = 8) mice were harvested. Two plates of cells of the same genotype were pooled into two groups for each genotype for microarray analysis. Cells were placed in TRIzol solution (Sigma, T9424) for extraction of total RNA according to the manufacturer’s instructions. 1 µg RNA was reverse transcribed to double-stranded cDNA, then transcribed into biotin-labeled cRNA according to the standard Affymetrix protocol. After fragmentation, 15 µg cRNA for each sample was hybridized for 16 h at 45 °C on a GeneChip Mouse Transcriptome Array 1.0 microarray, which was scanned at the UT Southwestern Microarray Core Facility. Array scanning was performed according to the manufacturer’s instructions (Affymetrix). Raw data were processed with Affymetrix Expression Console software for background correction and normalization.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary.

Data availability. All data supporting the findings of this study are available from the corresponding author upon request. Microarray data from this study have been deposited in the GenBank database under accession code GSE102955.

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### Experimental design

1. **Sample size**
   - Describe how sample size was determined.
   - No statistical methods were used to predetermine sample size.

2. **Data exclusions**
   - Describe any data exclusions.
   - For the epidermal tissue metabolome profiling, the Glut1fl/fl control group contained 8 mice, and Glut1fl/fl K14-Cre group contained 8 mice. One mouse in the Glut1fl/fl K14-Cre group showed inefficient Glut1 knockout as assessed by RT-PCR; thus, it was excluded from the data analysis.

3. **Replication**
   - Describe the measures taken to verify the reproducibility of the experimental findings.
   - For all the cell culture experiments, each experiment was repeated in at least 3 independent experiments. For primary cultured keratinocytes, each independent experiment used cells derived from different mice. All animal experiments (UV irradiation, wound healing, psoriasisform dermatitis) were repeated independently at least 3 times in different groups of mice. For gene expression analyses (microarray), samples were analyzed from a total of 8 mice, with RNA pooled from 2 independent mice. We confirmed the expression of genes of interest by RT-PCR in independent samples from at least 3 independent mice.

4. **Randomization**
   - Describe how samples/organisms/participants were allocated into experimental groups.
   - For primary cultured mouse keratinocytes, we isolated keratinocytes from littermates and determined the genotype of each cell line and confirmed the genotype by RT-PCR or Western blot after samples were collected or the experiments were completed. For mouse experiments, Glut1fl/fl females were crossed with Glut1fl/fl K14-Cre male which allowed for the generation of similar ratios of WT and conditional KO mice mice. Mice were gender and age matched for experiments. For experiments, gender and age matched mice were randomly allocated into groups for the experiments.

5. **Blinding**
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - For allocation into groups, treatments, and data collection, investigators were blinded to the genotypes of the mice. For all histological analyses, the rater was blinded to the genotype and/or treatment condition.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ☐   | ☒         |

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- 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 any assumptions or corrections, such as an adjustment for multiple comparisons
- Test values indicating whether an effect is present
  
  *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

## Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

- GraphPad Prism Version 7.0A
- MetaboAnalyst 3.0 (http://www.metaboanalyst.ca/)
- Affymetrix Expression Console Software (https://www.thermofisher.com/us/en/home/life-science/microarray-analysis/microarray-analysis-instruments-software-services/microarray-analysis-software/affymetrix-transcriptome-analysis-console-software.html)

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

## Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

- No unique materials were used in this study.
9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Glut1 (Thermo, RB-9052-P0, LOT #:9052P 1311L) Western Blot, both mouse and human samples in this study.
Glut1 (Millipore, 07-1401, LOT #: 2794923) western blot
Anti-Cytokeratin 10 antibody [EP1607HCY] (Abcam, ab76318, LOT #: GR202189-16) Western Blot and IHC, both mouse and human samples in this study.
Anti-Cytokeratin 14 antibody (Santa Cruz, SC-53253, LOT #: I0109) Western Blot and IHC, both mouse and human samples in this study.
Loricrin (BioLegend, 905104, LOT#: B243235). IHC, mouse samples in this study.
p-ACC Ser79 (CST, 3661. LOT#: 10) and p-S6 Ser240/244 (CST, 5364, LOT #: 6), Western Blot, both mouse and human samples in this study.
Ki-67 (Abcam, ab16667, LOT #: GR289011-2), IHC, mouse samples in this study.
CD45 (BioLegend 147701, LOT#: B181434), IHC, mouse samples in this study.
F4/80 (BioLegend 122602, LOT #: B226028) IHC, mouse samples in this study.
Gr-1 (BD Biosciences 550291, LOT #: 7152722), IHC, mouse samples in this study.
HSP90 (CST, 4877, LOT #: 4), Western Blot, both mouse and human samples in this study.
Involucrin (Thermo, MA5-11803, LOT #: RA2147064), Western Blot, human samples in this study.
ACC (CST, 3662, LOT #: 4), Western Blot, mouse samples in this study.
COX IV (CST, 4844, LOT #: 3), Western Blot, mouse samples in this study.
Secondary antibodies conjugated to HRP at a dilution of 1:2500 (Santa Cruz, Donkey anti-Rabbit: SC2077, Donkey anti-mouse: SC2096) For IHC and Western Blots in this study.
IF secondary antibody: Alex Fluor 546 goat-anti-rabbit or Alex Fluor 488 goat-anti-mouse secondary antibody (Life Technology, A11001, A11010). IF in this study.
Biotinylated Goat Anti-Rat IgG (Vector BA-9400, LOT #: ZB1216)
Biotinylated Goat Anti-Hamster IgG (Vector BA-9100, LOT #: ZB0702)
Antibodies were validated in house by Western blot and/or staining pattern. Antibodies performing as reported according to published literature (25, 30, 31, 34, 52) or the manufacturer’s description were used in the study.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Primary cultured mouse cell lines were harvested from the experimental mice around 1 week old. Primary cultured human keratinocytes were harvested from excess tissues obtained from excisions. HEK001 (ATCC CRL-2404) was purchased from ATCC. Squamous cell carcinoma cells (SCCT8) were provided by Andrew South.

b. Describe the method of cell line authentication used.

For the mouse/human primary cultured keratinocytes, we confirmed the cells by both the morphology and keratinocyte marker staining.

c. Report whether the cell lines were tested for mycoplasma contamination.

The cell lines were not tested for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

For animal experiments, we used C57BL/6 background mice, both male and female. Age and gender matched mice were used for experiments. Primary keratinocytes were derived from ~7 day old mice. For other experiments, all the mice were ~2 months old when experiments were initiated unless otherwise specified.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Human research participants were recruited from University of Texas Southwestern Medical Center outpatient clinics. Written, informed consent was obtained from all subjects. The study was approved by the UTSW institutional review board (STU 082010-241). Exclusion criteria included patients on subcutaneous and intravenous systemic immunosuppressant medications. Patients were clinically evaluated for psoriasis subtype and PASI score, and they completed a clinical questionnaire about demographics and current and past psoriasis treatments. 4-mm punch skin biopsy samples (from lesional skin) were procured. Human primary keratinocytes were pooled together for studies regardless of the gender/age.
Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

- **Data presentation**

  For all flow cytometry data, confirm that:

  1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  2. 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).
  3. All plots are contour plots with outliers or pseudocolor plots.
  4. A numerical value for number of cells or percentage (with statistics) is provided.

- **Methodological details**

  5. Describe the sample preparation.

      Cells were typically grown in 6 well in the indicated media, stained with 5 μM DCFDA for 30 min. Next, cells were washed, trypsinized, collected on ice, washed and resuspended in FACS buffer (DPBS, 2% FBS, and 0.2 mM EDTA).

  6. Identify the instrument used for data collection.

      BD FACSCalibur

  7. Describe the software used to collect and analyze the flow cytometry data.

      CellQuestPro was used to collect cells. FlowJo was used to analyze the data.

  8. Describe the abundance of the relevant cell populations within post-sort fractions.

      Not applicable

  9. Describe the gating strategy used.

      Not applicable

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