and faculty during the 2018 annual meeting, and invited a speaker to address challenges surrounding URM diversity in dermatology for a plenary talk during the 2019 annual meeting.

This year, the Board established a 5-year Diversity and Inclusion Committee to continue the work that was started by the ad hoc committee. This committee will work to foster diversity and inclusion across multiple aspects including gender, race, sexual orientation, and cognitive diversity in the programming of the annual meeting and in the membership.

**Racial diversity.** In addition to the Diversity and Inclusion Committee’s plans, the Society has several other initiatives that intend to foster the inclusion of URMs as members and speakers at the annual meeting. First, the Society established Freinkel Diversity Scholars, a fellowship program to increase the profile of women and minorities who are already members of SID and are committed to careers in investigative dermatology and cutaneous biology. Up to two individuals will be selected each year for fellowship awards to provide free registration at the SID annual meeting and a grant to support the travel activities to attend different International Societies for Investigative Dermatology member meetings over the following two years. More information can be found on the SID website (https://www.sidnet.org/page/Freinkel_Fellowships).

The Society has also established partnerships with the Student National Medical Association, which supports current and future URM medical students, and the American Academy of Dermatology, including the upcoming Diversity Champion Workshop. These partnerships intend to foster mentorship and training opportunities to promote diversity within the Society’s initiatives and membership.

The field of investigative dermatology has a rich history of rigorous science and strong mentorship. We are pleased with the progress in promoting more diversity within the Society’s membership and programming. Although gender and cognitive diversity have been prioritized in recent years, we believe that initiatives to promote inclusion of other types of diversity such as racial, sexual orientation, disability, and so forth, will improve our Society and investigative dermatology as a field.

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**CONFLICT OF INTEREST**

The authors state no conflict of interest.

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**REFERENCES**


**Exophilin-5 Supports Lysosome-Mediated Trafficking Required for Epidermal Differentiation**

**TO THE EDITOR**

Germline mutations in EXPH5 are associated with a recessive form of epidermolysis bullosa–related genes (McGrath et al., 2012; Pigors et al., 2014). Clinically identified mutations in EXPH5 result in premature truncations in the encoded protein exophilin-5 (also referred to as Slac2-b). This is associated with mild blistering, which in some cases is accompanied by mottled hypopigmentation. EXPH5 knockout mice are not available. The normal physiological role of exophilin-5 in epidermis and the mechanism by which its loss contributes to skin disease are unclear.

Exophilin-5, an effector of Rab27, is implicated in intracellular vesicular trafficking and secretion (Ostrowski and other established epidermolysis bullosa–related genes (McGrath et al., 2012; Pigors et al., 2014). Clinically identified mutations in EXPH5 result in premature truncations in the encoded protein exophilin-5 (also referred to as Slac2-b). This is associated with mild blistering, which in some cases is accompanied by mottled hypopigmentation. EXPH5 knockout mice are not available. The normal physiological role of exophilin-5 in epidermis and the mechanism by which its loss contributes to skin disease are unclear.

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Exophilin-5, an effector of Rab27, is implicated in intracellular vesicular trafficking and secretion (Ostrowski
Therefore, we hypothesized that exophilin-5 may be required in keratinocytes for the normal vesicular trafficking of lamellar bodies that extrude lipids into the extracellular space during normal epidermal differentiation. As lamellar bodies contain enzymes and membrane features associated with lysosomes, these secretory vesicles are also classified as lysosome-related organelles (LROs) (Eckhart et al., 2013; Raymond et al., 2008).

To define the role of exophilin-5 and lysosomal exocytosis in human epidermal homeostasis, we inhibited the LRO exocytosis trafficking pathway, both pharmacologically and genetically, in three-dimensional human organotypic skin cultures engineered using primary keratinocytes and devitalized human dermis (Ridky et al., 2010) (Supplementary Materials and Methods). The control tissues stratified and differentiated normally, as evidenced by the coordinated expression of keratin-10 and filaggrin (Figure 1a). However, epidermal differentiation was inhibited in the presence of vacuolin, a small molecule that blocks the fusion of secretory LROs with the plasma membrane (Figure 1a; Supplementary Figure S1). To specifically define the role of EXPH5 in lysosomal trafficking in keratinocytes and to test whether EXPH5 is required for normal epidermal homeostasis, we genetically depleted EXPH5 using two different short hairpin RNAs (Supplementary Figure S2). In contrast to the well-differentiated epidermis observed in the non-silenced short hairpin RNA control, tissues with EXPH5 depletion were poorly differentiated, as evidenced by loss of keratin-10 and filaggrin (Figure 1b Supplementary Figure S2). Consistent with the skin fragility phenotype associated with EXPH5 germline mutation, organotypic epidermis lacking EXPH5 was also hypoproliferative and lacked the uniform, peripheral distribution of desmoglein-3, which is seen throughout the keratinocyte plasma membranes in normal control samples (Supplementary Figure S3). Normal expression and localization of collagen VII and β1 integrin demonstrate that architecture at the dermal-epidermal junction was grossly intact in EXPH5-depleted tissue

Figure 1. Delivery of lysosome-related organelles to the plasma membrane is essential for normal keratinocyte differentiation. (a) Control organotypic human epidermis differentiated properly, including the spatially coordinated expression of keratin-10 (red) and filaggrin (green); nuclei (blue). Differentiation was inhibited when lysosome-mediated trafficking was blocked with vacuolin (10 μM). (b) Short hairpin RNA knockdown of exophilin-5 resulted in similarly diminished epidermal differentiation. Keratin-10 (red), filaggrin (green), and nuclei (blue). EXPH5i, exophilin-5 depletion; NS, non-silenced. Scale bars = 100 μm.
Together, these data demonstrate that normal epidermal differentiation and proliferation depend on EXPH5 and the delivery of LROs to the keratinocyte plasma membrane.

To determine whether secreted LROs from differentiating keratinocytes communicate with adjacent cells to promote differentiation, we assembled mosaic organotypic skin cultures containing an equal mixture of EXPH5-depleted and non-silenced keratinocytes. Epidermal differentiation was rescued in EXPH5-depleted keratinocytes when they were cocultured with non-silenced keratinocytes (tagged with K14-HA), as evidenced by the expression of both early and late differentiation proteins, including keratin-10, filaggrin, and loricrin (Figure 2; Supplementary Figure S4). To test whether this ability of normal keratinocytes to rescue differentiation in trans was specific to EXPH5 deficiency, we pharmacologically inhibited epidermal differentiation using Lys05, a lysosome inhibitor that we previously determined inhibits differentiation (Monteleon et al., 2018). As we observed with EXPH5 depletion, differentiation was also restored in Lys05-treated keratinocytes when cocultured with an equal number of normal keratinocytes (Supplementary Figure S5).

LRO-lamellar bodies contain a complex cargo and it is unlikely that rescue in trans results from the trafficking of a single factor. Lysosome-associated enzymes contribute to the biosynthesis of ceramide, formation of the cornified envelope, and the formation of the stratum corneum. To test whether the rescue in trans is mediated by a single factor, we assembled mosaic organotypic skin cultures containing an equal mixture of EXPH5-depleted and non-silenced keratinocytes. Epidermal differentiation was rescued in EXPH5-depleted keratinocytes when they were cocultured with non-silenced keratinocytes (tagged with K14-HA), as evidenced by the expression of both early and late differentiation proteins, including keratin-10, filaggrin, and loricrin (Figure 2; Supplementary Figure S4). To test whether this ability of normal keratinocytes to rescue differentiation in trans was specific to EXPH5 deficiency, we pharmacologically inhibited epidermal differentiation using Lys05, a lysosome inhibitor that we previously determined inhibits differentiation (Monteleon et al., 2018). As we observed with EXPH5 depletion, differentiation was also restored in Lys05-treated keratinocytes when cocultured with an equal number of normal keratinocytes (Supplementary Figure S5).

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proteolytic degradation that facilitates corneocyte desquamation (Egberts et al., 2004). It has also been demonstrated that secretory cargo initiates signaling back to keratinocytes through pathways that are not well understood (Appelqvist et al., 2013; Conus et al., 2012; Kovalenko et al., 2009). Interestingly, the signals that stimulate lysosomes to traffic as exocytic vesicles (Appelqvist et al., 2013; Jans et al., 2004), especially the sustained elevation of cytoplasmic calcium and increased oxidative stress, are the same factors responsible for initiating keratinocyte differentiation and, therefore, may be inextricably linked.

Many different physical, chemical, and genetic factors can lead to dysfunction in either lamellar body or LRO assembly or trafficking in keratinocytes. Disruption of normal lamellar bodies secretion compromises the lipid barrier, which renders skin more susceptible to dehydration, mechanical stress, and infiltration by microbes, and contributes to functional barrier defects in skin disorders, including eczema and ichthyosis (Elias and Wakefield, 2014; Milner et al., 1992; Rizzo et al., 2010; Werner et al., 1987). LROs are also necessary for the packaging and trafficking of melanin, which may be partially responsible for the mottled pigmentation in patients with EXPH5-epidermolysis bullosa (Turcan et al., 2016).

This current work furthers our understanding of epidermal homeostasis and suggests that keratinocyte differentiation within epidermis may not be a purely cell-autonomous process. Specifically, cell-cell communication via lysosome-mediated exocytosis may contribute to both early and late differentiation. The capacity for normal keratinocytes to rescue epithelial differentiation in trans, in adjacent keratinocytes with defective lysosomal exocytosis suggests that future gene therapy approaches for some genetic skin disorders may require correction of only a subset of keratinocytes rather than the entire epidermal keratinocyte population.

Data availability statement
The authors confirm that the data supporting the findings of this study are available in this article and its supplementary materials. Datasets related to this article can be found at https://doi.org/10.17632/82zk6kdx6c.1, hosted at Mendeleve (Lee [2019], “Role of Exophilin-5 in keratinocyte differentiation”, Mendeleve Data, v1).

CONFLICT OF INTEREST
The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS
Conceptualization: CLM, TWR; Data Curation: CLM, IYL, TWR; Formal Analysis: CLM, IYL, Funding Acquisition: TWR; Investigation: CLM, IYL; Methodology: CLM, TWR; Project Administration: CLM, TWR; Resources: CLM, TWR; Supervision: CLM, TWR; Validation: CLM, IYL; Visualization: CLM, IYL; Writing – Original Draft Preparation: CLM, TWR; Writing – Review and Editing: CLM, TWR, IYL

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**SUPPLEMENTARY MATERIAL**
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.04.014.

**REFERENCES**
SUPPLEMENTARY MATERIALS AND METHODS

Cell culture
All experiments were conducted using primary keratinocytes. Cells were isolated from normal human skin by previously described methods (Ridky et al., 2010). Keratinocytes were cultured in a 1:1 mixture of Gibco Keratinocytes-SFM medium + L-glutamine + EGF + BPE and Gibco Cascade Biologics 154 medium with 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA). Transduced keratinocytes were fully puromycin selected before the commencement of each experiment. Lys05 (Gift from R. Amavaradi at U. of Pennsylvania) was used at 2 μM. Vacuolin (Sigma, St. Louis, MO) was used at 2 μM. Ionomycin (Sigma) was used at 30 μM. LysoSensor (Molecular Probes, Carlsbad, CA) was used at 1 μM.

Lentiviral and Retroviral Production and Transduction
293T or 293T phoenix cells were cultured in Dulbecco’s Modified Eagles Medium (DMEM) supplemented with 5% FBS containing Antibiotic/Antimycotic. Lentiviral shRNA (OpenBiosystems, Lafayette, CO) particles were generated according to Thermo-scientific specifications and as described previously (Ridky et al., 2010). For the production of viral particles, lentiviral constructs were co-transfected with viral packaging plasmids pCMVΔR8.91 and pUC-MDG into 293T cells using Fu gene 6 Transfection Reagent (Promega, Fitchburg, WI). Retroviral particles, made from a phoenix cells carrying a LZRS viral vector expressing human K14 with a c-terminal HA tag, were used to transduce keratinocytes in order to label with HA.

Organotypic cultures
Organotypic skin cultures were established using parental or genetically engineered keratinocytes. For each culture, between 8.0 x 105 and 1.0 x 106 keratinocytes were suspended in 80 μL KGM or high calcium (1.2μM CaCl2) growth media, and seeded onto devitalized human dermis, according to previously established methods (Ridky et al., 2010). Unless otherwise indicated, small molecule and other chemical treatments were begun at seeding. OTCs were maintained at 37°C at an air-liquid interface for 4-12 days.

Immunofluorescence microscopy
Whole mount cryosections were prepared for immunofluorescence microscopy as previously described (Ridky et al., 2010). In short, slides were fixed in 4% paraformaldehyde or -20°C methanol, permeabilized as required and blocked with 10% horse serum/ PBS, followed by incubation with primary antibodies and secondary antibodies conjugated to fluorophores. Slides were mounted with Prolong Gold Antifade Reagent with DAPI (Life Technologies, Grand Island, NY). The primary antibodies used in this study were collagen-VII (Millipore, Burlington, MA), β1-integrin (Abcam, Cambridge, MA), ki67, desmoglein-3 (Thermo Fisher Scientific, Carlsbad, CA), HA, keratin-10, keratin-5, loricrin, and filaggrin (Covance, Dedham, MA). To quantify differentiation, the ratio of the area in pixels of keratin-10 positive epidermis to area in pixels of total epidermis was measured as a percentage in Imagel. This analysis was based previously reported methods (Billings et al., 2015, Natale et al., 2018). Results are the mean of at least 3 technical replicates across at least three biologic replicates from individual donors (± s.d.). Significance was assessed by Welch’s t-test across the biological replicates.

Quantitative RT/PCR
RNA was extracted from cells and tissues according to the RNeasy Mini Kit protocol (Qiagen, Valencia, CA), and reverse transcribed to cDNA using the High Capacity RNA-to cDNA kit (Applied Biosystems, Grand Island, NY). Quantitative PCR of resulting cDNA was conducted using Power SYBR Green Master Mix (Applied Biosystems, Grand Island, NY) and gene-specific primers, with three technical replicates on a ViiA 7 Real-Time PCR System (Life Technologies, Grand Island, NY). Relative expression was determined using the 2-[delta] [delta] Ct method. Results are the average from at least two individual donors (± s.d.).
Supplementary Figure S1. Vacuolin inhibits epidermal differentiation, the expression of differentiation markers, and lysosome-mediated traffic to the plasma membrane. (a) Epidermal keratinocyte differentiation in control and vacuolin treated organotypic cultures was determined by quantifying the area of keratin-10 positive epidermis relative to that of the total epidermis. Inhibition of lysosome-mediated exocytosis diminished epidermal differentiation (* indicates significance from Control, p < 0.05). (b) Quantitative PCR shows that vacuolin inhibits the calcium-induced expression of keratin-1 and filaggrin. (Data represents three independent experiments repeated with duplicates. *** indicates significance from control, p < 0.001. **** indicates significance from control, p < 0.0001.) (c) 2 μM Vacuolin, for 24-hours, blocked ionomycin-induced trafficking of lysosomes, shown with LysoSensor (green), to the plasma membrane (white arrows). Scale Bars = 50μm.
Supplementary Figure S2. shRNA mediated Exophilin-5 knockdown diminishes epidermal differentiation and lysosome-mediated traffic to the plasma membrane. (a) Differentiation in control and EXPH5i organotypic cultures was determined by quantifying the area of keratin-10 positive epidermis relative to the total epidermal area. Expophilin-5 depletion inhibited epidermal differentiation (* indicates significance from NS, p < 0.05 and ** indicates significance from NS, p < 0.01). (b) Exophilin-5 knockdown, and the associated decrease in calcium-induced Keratin-1 expression, was determined by qPCR. (Data represents at least two independent experiments repeated with duplicates. *** indicates significance from NS, p < 0.001). (c) EXPH5 depletion diminished ionomycin-induced peripheral lysosome trafficking (LysoSensor, green), to plasma membrane (white arrows). Scale bars = 50um.
Exophilin-5 depletion in organotypic epidermis inhibits proliferation and desmoglein localization, but does not grossly disrupt the basement membrane.

(a) The epidermal architecture in NS and EXPH5i organotypic cultures was evaluated by examining Keratin-5 (red), collagen-VII (green), (b) β1-integrin (red), collagen-VII (green), and (c) desmoglein-3 (green), and ki67 (red). Representative fields were analyzed for percentage of ki67 positive basal cells (*indicates significance from NS, p < 0.05).
Supplementary Figure S4. Expression of keratin-10 is restored in EXPH5i-keratinocytes co-cultured with normal NS control keratinocytes. (a) Organotypic epidermis engineered with non-silenced keratinocytes (tagged with K14-HA, green) differentiated normally (keratin-10, red). (b, c) EXPH5i OTCs were undifferentiated, lacking keratin-10 (red). (d, e) Mosaic tissues engineered from an equal mixture of control NS keratinocytes and EXPH5i keratinocytes expressed Keratin-10 (red) in both normal and EXPH5i keratinocytes. Scale bars = 100um.
Supplementary Figure S5. Lys05-induced block in epidermal differentiation is rescued in trans.
Keratinocytes were pre-treated with Lys05 (Monteleon et al., 2018) for 48 hours and then incorporated into OTCs, where they were incapable of differentiating as shown by the lack of (a) keratin-10, (b) filaggrin, and (c) loricrin expression. In contrast, mosaic OTCs engineered using Lys05-pretreated keratinocytes mixed with normal keratinocytes (tagged with HA, green), differentiated normally. Scale bars = 100um.